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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>7</sup> : C12N 15/54, 9/12, A61K 31/70		A2	(11) International Publication Number: <b>WO 00/52173</b>
			(43) International Publication Date: 8 September 2000 (08.09.00)
(21) International Application Number: PCT/CA00/00223		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 2 March 2000 (02.03.00)			
(30) Priority Data: 60/122,516 2 March 1999 (02.03.99) US			
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(54) Title: CLONED HUMAN SPHINGOSINE KINASE HOMOLOGUES			
(57) Abstract			
<p>The present invention provides newly identified and isolated polynucleotides and their polypeptides and their uses and in particular to newly identified and isolated polynucleotides and polypeptides of the sphingosine kinase family. Three isolated polynucleotides and polypeptides for three human SK homologues are described: SKA, SKB and SKC.</p>			

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## Cloned Human Sphingosine Kinase Homologues

### Field of Invention

The present invention is related to the field of molecular biology. In particular  
5 the present invention is related to newly identified and isolated polynucleotides and  
their polypeptides and their uses and in particular to newly identified and isolated  
polynucleotides and polypeptides of the sphingosine kinase family.

### Background

10 Sphingolipids are complex structural lipids which are found in membranes.  
One of the more prominent sphingolipids is sphingomyelin. Sphingomyelin is  
hydrolyzed by sphingomyelinase to form ceramide which in turn is metabolized by  
ceramidase to form sphingosine. Sphingosine kinase (SK) is the enzyme which  
phosphorylates sphingosine to form sphingosine 1-phosphate (S1P) and thereby SK in  
15 effect controls S1P production.

It has been suggested that S1P plays an intracellular role in cell proliferation  
and inhibits and/or blocks apoptosis, among other things. S1P also acts as an  
endogenous ligand for certain G-protein coupled receptors, including the edg-3  
20 receptor. It has also been found that SK inhibitors block thrombin signalling  
pathways and induce apoptosis.

Accordingly, in view of the role of SK and S1P in key pathways, it would be  
desirable to have the cloned human SK homologues for use as drug targets. In  
25 particular, using the human SK homologues, SK inhibitors could be determined  
which inhibitors could be used in anti-proliferative diseases: cancer, psoriasis,  
reactive gliosis; or to suppress inappropriate cell survival and to block inflammatory  
S1P production in neurodegenerative diseases, demyelinating diseases, asthma, and  
allergies

30

### Summary of the Invention

The present invention provides the isolated polynucleotides and polypeptides for the human SK homologues. In particular, the present invention provides three  
5 isolated polynucleotides and polypeptides for the three human SK homologues: SKA; SKB; and SKC; and variants thereof.

In accordance with an aspect of the present invention there is provided isolated polypeptides for the human SK homologues SKA, SKB and SKC comprising  
10 the sequences as set out in Figures 3, 6 and 9, respectively, and variants thereof.

In accordance with another aspect of the present invention there is provided isolated polynucleotides of SKA, SKB and SKC comprising the sequences as illustrated in Figures 2, 5 and 8, respectively, and variants thereof.  
15

In accordance with a further aspect of the present invention there are provided isolated polynucleotides encoding human SKA, SKB and SKC, including mRNAs, cDNAs, genomic DNAs. In addition, embodiments of the invention include diagnostic, prophylactic, clinical or therapeutical useful variants of these isolated  
20 nucleotide sequences SKA, SKB and SKC and compositions thereof. Also included in an aspect of the invention are naturally occurring allelic variants of SKA, SKB and SKC and polypeptides encoded thereby.

In accordance with another aspect of the invention, there are provided  
25 methods for producing the polypeptides for SKA, SKB and SKC and for determining inhibitors to such polypeptides, including antibodies.

In accordance with another aspect there are polynucleotides that hybridize to SKA, SKB and SKC nucleotide sequences, particularly under stringent conditions.  
30

In accordance with yet another aspect of the invention, there are provided methods for identifying compounds which interact with the polypeptide or polynucleotide of SKA, SKB or SKC.

- 5           There are also provided compositions comprising a polypeptide or polynucleotide of SKA, SKB or SKC for administration to a cell or to a multicellular organism.

10   **Description of the Figures**

Figure 1 is an illustration of the full length nucleotide sequence of the cDNA of human SKA.

- 15           Figure 2 is an illustration of the nucleotide sequence of the coding region of SKA.

Figure 3 is an illustration of the predicted amino acid sequence of SKA as illustrated in Figure 2.

20

Figure 4 is an illustration of the full length nucleotide sequence of the cDNA of human SKB.

- 25           Figure 5 is an illustration of the nucleotide sequence of the coding region of SKB.

Figure 6 is an illustration of the predicted amino acid sequence of SKB as illustrated in Figure 5.

- 30           Figure 7 is an illustration of the full length nucleotide sequence of the cDNA of human SKC.

Figure 8 is an illustration of the nucleotide sequence of the coding region of SKC.

5        Figure 9 is an illustration of the predicted amino acid sequence of SKC as illustrated in Figure 8.

Figure 10 is an illustration of the alignment of the amino acid sequences of human SKA, SKB and SKC.

10

Figure 11 is an illustration of the results of the phosphorylation assays exemplified in Example 4 for SKA, SKB and SKB.

## **Detailed Description**

15

### **Definitions**

The following definitions are used herein for the purpose of describing particular terms used in the application. Any terms not specifically defined should be given the meaning commonly understood by one of ordinary skill in the art to which the invention pertains.

20

“Biologically Active” refers to those forms, fragments, or domains of any sphingosine kinase polypeptide which retain at least some of the biological and/or antigenic activities of a naturally occurring sphingosine kinase.

25

“Chimeric” molecules may be constructed by introducing all or part of the nucleotide sequence of this invention into a vector containing additional nucleic acid sequence which might be expected to change any one (or more than one) of the following characteristics: cellular location, distribution, ligand-binding affinities, interchain affinities, degradation/turnover rate, signaling, etc.

30

“Derivative” refers to those amino acid sequences and nucleotide sequences which have been chemically modified. Such techniques for polypeptide derivatives include: ubiquitination; labeling (see above); pegylation (derivatization with polyethylene glycol); and chemical insertion or substitution of amino acids such as ornithine which do not normally occur in human proteins. A nucleotide sequence derivative would encode an amino acid which retains its essential biological activity and characteristics of the natural molecule.

10 As used herein “human sphingosine kinase” refers to the isolated polypeptide or polynucleotide sequences of the different isoforms of human sphingosine kinase, including human SKA, human SKB and human SKC, in either naturally occurring or synthetic form.

15 As used herein, “human sphingosine kinase A” or “human SKA” refers to the polynucleotide or polypeptide of an isoform of human sphingosine kinase as illustrated by the sequences of Figure 2 and 3, respectively, and by polypeptide sequences which preferably have at least 85% sequence identity with each other and Figure 3, and more preferably at least 90% sequence identity with each other and  
20 Figure 3, and most preferably at least 95% sequence identity with each other and Figure 3, or polynucleotide sequences which encode such polypeptide sequence identities.

As used herein, “human sphingosine kinase B” or “human SKB” refers to the  
25 polynucleotide or polypeptide of an isoform of human sphingosine kinase as illustrated by the sequences of Figure 5 and 6, respectively, and to the polypeptide sequences which preferably have at least 85% sequence identity with each other and Figure 6, and more preferably at least 90% sequence identity with each other and Figure 6, and most preferably at least 95% sequence identity with each other and Figure 6 or  
30 polynucleotide sequences which encode such polypeptide sequence identities.

As used herein, "human sphingosine kinase C" or "human SKC" refers to the polynucleotide or polypeptide of an isoform of human sphingosine kinase as illustrated by the sequences of Figure 8 and 9, respectively, and by the polypeptide sequences which preferably have at least 85% sequence identity with each other and  
5 Figure 9, and more preferably at least 90% sequence identity with each other and Figure 9, and most preferably at least 95% sequence identity with each other and Figure 9 or polynucleotide sequences which encode such polypeptide sequence identities.

10 "Inhibitor" is any substance which retards or prevents a biochemical, cellular or physiological reaction or response. Common inhibitors include but are not limited to antisense molecules, antibodies, and antagonists.

"Insertions" or "deletions" are typically in the range of about 1 to 5 amino  
15 acids and do not result in a change in biological activity of the polypeptide. The variation allowed may be experimentally determined by producing the peptide synthetically or by systematically making insertions, deletions, or substitutions of nucleotides in the human sphingosine kinase sequence using recombinant DNA techniques.

20 As used herein "isolated" means separated from nucleotide sequences that encode other proteins or from other peptides. For example, a polypeptide or polynucleotide naturally present in a living organism is not "isolated" but when separated from the coexisting nucleotides/peptides it is "isolated".

25 "Nucleotide sequences" as used herein are oligonucleotides, polynucleotides, and fragments or portions thereof, and are DNA or RNA of genomic or synthetic origin which may be single or double stranded, and represent the sense or complement or antisense strands.

30 An "oligonucleotide" is a stretch of nucleotide residues, which has a



sufficient number of bases to be used as an oligomer, amplimer or probe in a polymerase chain reaction (PCR). Oligonucleotides are prepared from genomic or cDNA sequence and are used to amplify, reveal or confirm the presence of a similar DNA or RNA in a particular cell or tissue. Oligonucleotides or oligomers comprise  
5 portions of a DNA sequence having at least about 10 nucleotides and as many as about 35 nucleotides, preferably about 25 nucleotides.

An "oligopeptide" is a short stretch of amino acid residues and may be expressed from an oligonucleotide. It may be functionally equivalent to and the same  
10 length as (or considerably shorter than) a "fragment", "portion", or "segment" of a polypeptide. Such sequences comprise a stretch of amino acid residues of at least about 5 amino acids and often about 17 or more amino acids, typically at least about 9 to 13 amino acids, and of sufficient length to display biological and/or antigenic activity.

15

As used herein "purified" refers to amino acid sequences that are removed from their natural environment, and are isolated or separated, and are at least 60% free, preferably at least 75 % free, and most preferably at least 90% free from other components with which they are naturally associated.

20

A "portion" or "fragment" of a nucleotide or nucleic acid sequence comprises all or any part of the sequence having fewer nucleotides than about 6 kb, preferably fewer than about 1 kb. A portion or fragment can be used as a probe. Such probes may be labeled with reporter molecules using nick translation, Klenow fill-in  
25 reaction, PCR or other methods well known in the art. To optimize reaction conditions and to eliminate false positives, nucleic acid probes may be used in Southern, Northern or in situ hybridizations to determine whether DNA or RNA encoding spingosine kinase is present in a cell type, tissue, or organ.

30

"Probes" may be derived from naturally occurring, recombinant, or chemically synthesized single - or double - stranded nucleic acids or be chemically

synthesized. They are useful in detecting the presence of identical or similar sequences.

5       “Reporter” molecules are those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents which associate with, establish the presence of, and may allow quantification of a particular nucleotide or amino acid sequence.

10       A “signal or leader sequence” can be used, when desired, to direct the polypeptide through a membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous sources by recombinant DNA techniques.

15       Amino acid “substitutions” are conservative in nature when they result from replacing one amino acid with another having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine.

20       “Standard” is a quantitative or qualitative measurement for comparison. It is based on a statistically appropriate number of normal samples and is created to use as a basis of comparison when performing diagnostic assays, running clinical trials, or following patient treatment profiles.

25       “Stringent conditions” is used herein to mean conditions that allow for hybridization of substantially related nucleic acid sequences. Such hybridization conditions are described by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, 1989. Generally, stringency occurs within a range from about 5 °C below the melting temperature of the probe to about 20 °C – 25 °C below the melting temperature. As understood by ordinary skilled  
30       persons in the art, the stringency conditions may be altered in order to identify or detect identical or related nucleotide sequences. Factors such as the length and nature

(DNA, RNA, base composition) of the sequence, nature of the target (DNA, RNA, base composition, presence in solution or immobilization, etc.) and the concentration of the salts and other components (e.g. the presence or absence of formamide, dextran sulfate and/or polyethylene glycol) are considered and the hybridization  
5 solution may be varied to generate conditions of either low or high stringency.

“Sequence Identity” is known in the art, and is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences, particularly, as determined by the match between strings  
10 of such sequences. Sequence identity can be readily calculated by known methods (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence*  
15 *Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two sequences, the term is well known to skilled artisans (see, for example, *Sequence Analysis in Molecular Biology*; *Sequence Analysis Primer*; and Carillo, H., and  
20 Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988)). Methods commonly employed to determine identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988) or, preferably, in Needleman and Wunsch, *J. Mol. Biol.*, 48: 443-445, 1970, wherein the parameters are as set in version 2 of DNASIS (Hitachi Software  
25 Engineering Co., San Bruno, CA). Computer programs for determining identity are publicly available. Preferred computer program methods to determine identity between two sequences include, but are not limited to, GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. *et al.*, *J. Molec. Biol.* 215: 403-410 (1990)). The  
30 BLASTX program is publicly available from NCBI ([blast@ncbi.nlm.nih.gov](mailto:blast@ncbi.nlm.nih.gov)) and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD

20894; Altschul, S., *et al.*, *J. Mol. Bio.* 215: 403-410 (1990)). Computational Molecular Biology, Lesk, A.M, ed. Unless specified otherwise in the claims, the percent identity for the purpose of interpreting the claims shall be calculated by the Needleman and Wucnsch algorithm with the parameters set in version 2 of DNASIS.

5

"Variants" are polynucleotides or polypeptides that differ from a reference polynucleotide or polypeptide, respectively, but retain essential properties of the reference, preferably, in the case of polypeptides the variant retains the biological activity of the naturally occurring polypeptide. A typical variant of a polynucleotide differs in nucleotide sequence from another reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequences of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, insertions and deletions in the polypeptide encoded by the reference sequences, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, insertions and deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques, by direct synthesis, and by other recombinant methods known to skilled artisans.

25

### Description

The invention relates to novel polypeptides and polynucleotides for human sphingosine kinase as described in greater detail below. The invention particularly relates to the three sphingosine kinase homologues: human SKA, human SKB and human SKC. More particularly, human SKA, SKB and SKC having the nucleotide

30

sequences as set out in Figures 2, 5 and 8 for SKA, SKB and SKC, respectively, and variants thereof are provided for herein.

In addition, the polypeptides of the invention include the polypeptides  
5 comprising the sequences as set out in Figures 3, 6 and 9 as well as variants of these polypeptides, particularly variants which retain the biological activity of the naturally occurring sphingosine kinase.

Fragments of the polypeptides of the invention may be employed for  
10 producing the corresponding full length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full length polypeptides of the invention.

The polynucleotides comprising sequences encoding human SKA, SKB and  
15 SKC (or their complement) and variants thereof have numerous applications in techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use in the construction of oligomers for PCR, use for chromosome and gene mapping, use in the recombinant production of SKA, SKB and SKC, and use in generation of antisense DNA or RNA, their chemical analogs  
20 and the like. Uses of nucleotides encoding SKA, SKB and SKC disclosed herein are exemplary of known techniques and are not intended to limit their use in any technique known to a person of ordinary skill in the art. Furthermore, the nucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide  
25 sequences that are currently known, e.g., the triplet genetic code, specific base pair interactions, etc.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of human SKA, SKB and SKC encoding  
30 nucleotide sequences may be produced. Some of these will only bear minimal homology to the nucleotide sequence of the known and naturally occurring SKA,

SKB and SKC. The invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring SKA, SKB and SKC, and all such variations are to be considered as being specifically disclosed.

Although the nucleotide sequences which encode SKA, SKB and SKC, their derivatives or their variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring SKA, SKB and SKC, respectively, under stringent conditions, it may be advantageous to produce nucleotide sequences encoding SKA, SKB and SKC or its derivatives possessing a substantially different codon usage. Codons can be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding SKA, SKB and SKC and/or its derivatives without altering the encoded amino acid sequence include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

Human genes often show considerable actual polymorphism; that is, variation in nucleotide sequence among a fraction of the entire human population. In many cases this polymorphism can result in one or more amino acid substitutions. While some of these substitutions show no demonstrable change in function of the protein, others may produce varying degrees of functional effects. In fact, many natural or artificially produced mutations can lead to expressible human SK proteins. Each of these variants, whether naturally or artificially produced, is considered to be equivalent and specifically incorporated into the present invention.

Nucleotide sequences encoding human SKA, SKB and SKC may be joined to a variety of other nucleotide sequences by means of well established recombinant

DNA techniques (Sambrook J et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor NY; or Ausubel FM et al (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York City).

Useful nucleotide sequences for joining to human SK include an assortment of  
5 cloning vectors such as plasmids, cosmids, lambda phage derivatives, phagemids, and  
the like. Vectors of interest include expression vectors, replication vectors, probe  
generation vectors, sequencing vectors, etc. In general, vectors of interest may  
contain an origin of replication functional in at least one organism, convenient  
restriction endonuclease sensitive sites, and selectable markers for one or more host  
10 cell systems.

Human SK specific hybridization probes are capable of hybridizing with  
naturally occurring nucleotide sequences encoding human SKA, SKB and SKC.  
Such probes may also be used for the detection of similar sequences and should  
15 preferably contain at least 60% nucleotide identity to SK sequence. The  
hybridization probes of human SK may be derived from the nucleotide sequence  
presented in the Figures for the full length sequence for SKA, SKB and SKC, namely,  
Figures 1, 4 and 7, respectively, or from genomic sequences including promoter,  
enhancers, introns or 3'-untranslated regions of the native gene. Hybridization probes  
20 may be labeled by a variety of reporter molecules using techniques well known in the  
art. Preferably, the hybridization probes incorporate at least 15 nucleotides, and  
preferably at least 25 nucleotides, of the SK protein. Suitable hybridization probes  
would include: consensus fragments, for example, those regions of the human SK  
isoforms that are identical, as particularly exemplified in Figure 10.

25

It will be recognized that many deletional or mutational analogs of nucleic  
acid sequences for human SK will be effective hybridization probes for human SK  
nucleic acid. Accordingly, the invention relates to nucleic acid sequences that  
hybridize with such SK encoding nucleic acid sequences under stringent conditions.

30

Stringent conditions will generally allow hybridization of sequence with at

least about 70% sequence identity, more preferably at least about 80-85% sequence identity, even more preferably at least about 90% sequence identity, and most preferably with at least about 95% sequence identity. Hybridization conditions and probes can be adjusted in well-characterized ways to achieve selective hybridization of human-derived probes. Nucleic acid molecules that will hybridize to human SK encoding nucleic acid under stringent conditions can be identified functionally, using methods outlined above, or by using for example the hybridization rules reviewed in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Press, 1989. Without limitation, examples of the uses for hybridization probes include: histochemical uses such as identifying tissues that express human SK; measuring mRNA levels, for instance to identify a sample's tissue type or to identify cells that express abnormal levels of human SK; and detecting polymorphisms in the human SK. RNA hybridization procedures are described in Maniatis et al. *Molecular Cloning, a Laboratory Manual* (Cold Spring Harbor Press, 1989). PCR as described US Patent No's. 4,683,195; 4,800,195; and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequence which encodes the human SK sequences of the invention. Such probes used in PCR may be of recombinant origin, chemically synthesized, or a mixture of both. Oligomers may comprise discrete nucleotide sequences employed under optimized conditions for identification of human SK in specific tissues or diagnostic use. The same two oligomers, a nested set of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for identification of closely related DNA's or RNA's. Rules for designing PCR primers are now established, as reviewed by PCR Protocols, Cold Spring Harbor Press, 1991. Degenerate primers, i.e., preparations of primers that are heterogeneous at given sequence locations, can be designed to amplify nucleic acid sequences that are highly homologous to, but not identical to human SK. Strategies are now available that allow for only one of the primers to be required to specifically hybridize with a known sequence. See, Froman et al., *Proc. Natl. Acad. Sci. USA* 85: 8998, 1988 and Loh et al., *Science* 243: 217, 1989. For example, appropriate nucleic acid primers can be ligated to the nucleic acid sought to be amplified to provide the hybridization partner for one of the primers. In this way, only one of the primers



need be based on the sequence of the nucleic acid sought to be amplified. PCR methods of amplifying nucleic acid will utilize at least two primers. One of these primers will be capable of hybridizing to a first strand of the nucleic acid to be amplified and of priming enzyme-driven nucleic acid synthesis in a first direction.

5 The other will be capable of hybridizing the reciprocal sequence of the first strand (if the sequence to be amplified is single stranded, this sequence will initially be hypothetical, but will be synthesized in the first amplification cycle) and of priming nucleic acid synthesis from that strand in the direction opposite the first direction and towards the site of hybridization for the first primer. Conditions for conducting such

10 amplifications, particularly under preferred stringent hybridization conditions, are well known. See, for example, PCR Protocols, Cold Spring Harbor Press, 1991.

Other means of producing specific hybridization probes for human SK include the cloning of nucleic acid sequences encoding human SK or human SK variants or

15 derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate reporter molecules.

20 It is possible to produce a DNA sequence, or portions thereof, entirely by synthetic chemistry. After synthesis, the nucleic acid sequence can be inserted into any of the many available DNA vectors and their respective host cells using techniques which are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into the nucleotide sequence. Alternately, a portion of

25 sequence in which a mutation is desired can be synthesized and recombined with longer portion of an existing genomic or recombinant sequence.

The nucleotide sequence for human SK can be used in an assay to detect inflammation or disease associated with abnormal levels of SK expression. The

30 cDNA can be labeled by methods known in the art, added to a fluid, cell or tissue sample from a patient, and incubated under hybridizing conditions. After an

incubation period, the sample is washed with a compatible fluid which optionally contains a reporter molecule. After the compatible fluid is rinsed off, the reporter molecule is quantitated and compared with a standard as previously defined.

- 5           A diagnostic test for aberrant expression of SK can accelerate diagnosis and proper treatment of abnormal conditions of SK activity.

          New nucleotide sequences can be assigned to chromosomal subregions by physical mapping. The mapping of new genes or nucleotide sequences provide useful  
10 landmarks for investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome, such as ataxia telangiectasia (AT), has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 1 1q22-23 (Gatti et al (1988) Nature 336:577-580), any sequences mapping to that area may represent or reveal genes for further  
15 investigation. The nucleotide sequence of the subject invention may also be used to detect differences in gene sequence between normal and carrier or affected individuals.

          Nucleotide sequences encoding human SK may be used to produce a purified  
20 oligo - or polypeptide using well known methods of recombinant DNA technology. Goeddel (1990, Gene Expression Technology, Methods and Enzymology, Vol. 185, Academic Press, San Diego CA) is one among many publications which teach expression of an isolated nucleotide sequence. The oligopeptide may be expressed in a variety of host cells, either prokaryotic or eukaryotic. Host cells may be from the  
25 same species from which the nucleotide sequence was derived or from a different species. Advantages of producing an oligonucleotide by recombinant DNA technology include obtaining adequate amounts of the protein for purification and the availability of simplified purification procedures.

30           Cells transformed with DNA encoding human SK may be cultured under conditions suitable for the expression of human kinases and recovery of such peptides

from cell culture. Human SK produced by a recombinant cell may be secreted or may be contained intracellularly, depending on the particular genetic construction used. In general, it is more convenient to prepare recombinant proteins in secreted form.

Purification steps vary with the production process and the particular protein produced. Often an oligopeptide can be produced from a chimeric nucleotide sequence. This is accomplished by ligating the nucleotides from human SK or a desired portion of the polypeptide to a nucleic acid sequence encoding a polypeptide domain which will facilitate protein purification (Kroll DJ et al (1993) DNA Cell Biol. 12:441-53).

10

In addition to recombinant production, fragments of human SK may be produced by direct peptide synthesis using solid-phase techniques (e.g. Stewart et al (1969) Solid-Phase Peptide Synthesis, WH Freeman Co., San Francisco QA; Merrifield J (1963) J Am Chem. Soc. 85:2149-2154). Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Foster City, CA) in accordance with the instructions provided by the manufacturer. Additionally, a particular portion of human SK may be mutated during direct synthesis and combined with other parts of the peptide using chemical methods.

20

Human SK for antibody induction does not require biological activity; however, the protein must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acids, preferably at least 10 amino acids. They should mimic a portion of the amino acid sequence of the protein and may contain the entire amino acid sequence. An antigenic portion of human SK may be fused to another protein such as keyhole limpet hemocyanin, and the chimeric molecule used for antibody production.

25

Antibodies specific for human SK may be produced by inoculation of an appropriate animal with the polypeptide or an antigenic fragment. An antibody is specific for human SK if it is produced against an epitope of the polypeptide and binds to at least part of the natural or recombinant protein. Antibody production

30

includes not only the stimulation of an immune response by injection into animals, but also analogous processes such as the production of synthetic antibodies, the screening of recombinant immunoglobulin libraries for specific- binding molecules (e.g. Orlandi R et al (1989) PNAS 86:3833-3837, or Huse WD et al (1989) Science 5 256:1275-1281) or the in vitro stimulation of lymphocyte populations. Current technology (Winter G and Mistein C (1991) Nature 349:293-299) provides for a number of highly specific binding reagents based on the principles of antibody formation. These techniques may be adapted to produce molecules which specifically bind SK.

10

An additional embodiment of the subject invention is the use of human SK specific antibodies, inhibitors, ligands or their analogs as bioactive agents to treat inflammation or disease possibly including, but not limited to viral, bacterial or fungal infections; allergic responses; mechanical injury associated with trauma; 15 hereditary diseases; lymphoma or carcinoma; or other conditions which activate the genes of kidney, lung, heart, lymphoid or tissues of the nervous system.

Bioactive compositions comprising agonists, antagonists, receptors or inhibitors of human SK may be administered in a suitable therapeutic dose 20 determined by any of several methodologies including clinical studies on mammalian species to determine maximal tolerable dose and on normal human subjects to determine safe dose. Additionally, the bioactive agent may be complexed with a variety of well established compounds or compositions which enhance stability or pharmacological properties such as half-life. It is contemplated that the therapeutic, 25 bioactive composition may be delivered by intravenous infusion into the bloodstream or any other effective means which could be used for treating problems involving aberrant expression of the EDG-7 gene.

The examples below are provided to illustrate the subject invention. These 30 examples are provided by way of illustration and are not included for the purpose of limiting the invention.

**Example 1****Cloning of PSKA****5 A. Diagnostic PCR of various templates for the presence of human SKA cDNA**

The following pairs of primers were designed:

**10 5' end Primers**

SK1F 5' AAC CCG CGC GGC GCA AGG GCA AGG C 3'

SK2F 5' AAG GGC AAG GCC TTG CAG CTC TTC C 3'

**3' end Primers**

**15 SK1R 5' CAG GCC GCT CCA TGA GCC CGT TCA C 3'**

SK2R 5' GCA TCA GCC CGT CTC CAG ACA TGA 3'

Using these primers, PCR was conducted under the conditions as set out below on the templates from the following sources:

**20**

Template source DNA of cDNA Libraries prepared from Human Lung Fibroblasts WI-38 (Origene Technologies Inc., Cat. No. DLH-102), Human Liver (Origene Technologies Inc., Cat. No. DLH-100), cultured human Jurkat T-cells (Origene Technologies Inc., Cat. No. DLH-115), HeLa cultured cells (Origene Technologies Inc., Cat. No. DLH-103), Human kidney proximal tubules (ATCC), HeLa cultured cells (Invitrogen, Cat. No. A550-26), Human Lung (Clonotech, Cat. No. 7114-1), HeLa cultured cells (Clonotech, Cat. No. HL5013a), human small intestine (Clonotech, Cat. No. HL1133a). Each template was amplified with each pair of primers under the following condition of PCR amplification by using Expand TM

**25**

**30** PCR kit of Boehringer Mannheim (Catalogue no. 1681-842).

Each reaction contained the following reagents:

2  $\mu$ l of 10x PCR Buffer 3

0.4  $\mu$ l of 25mM dNTP mix

0.6  $\mu$ l of Primer SKF1 or SKF2 (10pm/ $\mu$ l)

5 0.6  $\mu$ l of Primer SKR1 or SKR2 (10pm/ $\mu$ l)

0.3 $\mu$ l of Enzyme (3unit)

15.1  $\mu$ l water

1  $\mu$ l DNA

10 PCR conditions:

Incubate: 94°C for 2 min

30 cycles: 94°C for 1 min

62°C for 1 min

68°C for 30 sec

15 Incubate: 68°C for 8 min

Hold: 4°C

A 200 bp (approximately) DNA fragment was amplified from all templates except

20 Origene's HeLa cDNA library and Clontech's small intestine cDNA library.

The cDNA library from cultured HeLa cells (Invitrogen) appeared to contain PSKA clones.

## 25 B. PCR Screening of HeLa cDNA Library

10 000 (10K) clone pools from cDNA library from cultured HeLa cells (Invitrogen,

Cat. No. A550-26): Approximately, ten thousand clones were grown on an agar plate, scraped and re-suspended in one ml of 2X YT + 20% glycerol. Overall,

30 610 pools (10K) were prepared. Equal proportions of twelve 10K pools were mixed to prepare 120K pools. In all, there were fifty one 120K pools. All pools are kept as

frozen stocks at 80°C. For PCR screening, a small portion of frozen stock was re-suspended in 100 µl of 2X YT + 20% glycerol and used as template.

### C. Screening of 120K and 10K bacterial

5

All fifty one 120K bacterial pools and 10K pools of positive 120K pools were amplified under the following condition of PCR amplification by using Expand™ PCR system from Boehringer Mannheim (Catalogue no. 1681-842) with the following pair of primers.

10

SK1F        5' AAC CCG CGC GGC GCA AGG GCA AGG C  
SK1R        5' CAG GCC GCT CCA TGA GCC CGT TCA C 3'

Each reaction contained the following reagents:

15

2 µl of 10x PCR Buffer 3  
0.4 µl of 25mM dNTP mix  
0.6 µl of Primer SKF1 (10pm/µl)  
0.6 µl of Primer SKR1 (10pm/µl)  
20 0.3 µl of Enzyme (3unit)  
15.1 µl water  
1 µl DNA

PCR conditions:

25 Incubate:            94°C for 2 min  
30 cycles:            94°C for 40 sec  
                         60°C for 40 sec  
                         68°C for 40 sec  
Incubate:            68°C for 8 min  
30  
Hold:                4°C

Majority of 120K bacterial pools was found positive indicating that PSKA is an abundantly expressed gene. Four 10 K pools (62, 64, 74, 403, and 404 from selected positive 120K pools) were found positive.

5

**D. PCR screening of sub-pools of 10K pool #403:**

The bacterial colonies were grown from the positive 10K pool #403 on the agar plate. Plugs containing 300 – 1000 bacterial colonies were lifted from the agar plate. The  
10 bacterial colonies were re-suspended into 500 ul of 2XYT + 20% glycerol. The bacterial re-suspensions were used as template to amplify with the following pair of primers.

SK1F            5' AAC CCG CGC GGC GCA AGG GCA AGG C  
15 SK1R            5' CAG GCC GCT CCA TGA GCC CGT TCA C 3'

Each reaction contained the following reagents:

2 µl of 10x PCR Buffer 3  
20 0.4 µl of 25mM dNTP mix  
0.6 µl of Primer SKF1 (10pm/µl)  
0.6 µl of Primer SKR1 (10pm/µl)  
0.3 µl of Enzyme (3unit)  
15.1 µl water  
25 1 µl DNA

PCR conditions:

Incubate:            94°C for 2 min  
30 cycles:            94°C for 40 sec  
30                      60°C for 40 sec  
                          68°C for 40 sec



Incubate: 68°C for 8 min

Hold: 4°C

- 5 Several positive sub-pools were identified. Sub-pool #403-42 was used further to isolate PSKA clone.

#### **E. Hybridization screening of sub-pool # 403-42**

- 10 Bacterial colonies were grown from sub-pool #403-42 on the agar plate and were transferred to nylon filters. Filter hybridization was carried out using 200 bp DNA fragment (amplified by SK1F and SK1R from template 10K pool no. 62) as probe. The following hybridization conditions were employed:

- 15 5X SSPE

5X Denharts solution (1% Ficoll, 1% Polyvinylpyrrolidone, 1% BSA)  
25µg/ml fish sperm DNA

Hybridise at 65°C overnight.

20

The filters were washed 2 times in 2X SSPE and 0.1% SDS at room temperature for 30 minutes each, then 2 times in 2XSSPE and 0.1% SDS at 50°C for 20 minutes each and finally two times in 0.1XSSPE and 0.1% SDS.

- 25 One positive plug 403-42.1 was identified.

#### **F. PCR screening of plug #403-42.1**

- 30 Bacterial colonies from plug #403-42.1 were grown on the agar plate, picked and re-suspended into 100 µl of 2XYT + 20% glycerol. The bacterial re-suspensions were used as template to amplify with the following pair of primers.

SK1F        5' AAC CCG CGC GGC GCA AGG GCA AGG C  
SK1R        5' CAG GCC GCT CCA TGA GCC CGT TCA C 3'

5 Each reaction contained the following reagents:

2 µl of 10x PCR Buffer 3  
0.4 µl of 25mM dNTP mix  
0.6 µl of Primer SKF1 (10pm/µl)  
10 0.6 µl of Primer SKR1 (10pm/µl)  
0.3 µl of Enzyme (3unit)  
15.1 µl water  
1 µl DNA

15 PCR conditions:

Incubate:        94°C for 2 min  
30 cycles:        94°C for 40 sec  
                    60°C for 40 sec  
                    68°C for 40 sec  
20 Incubate:        68°C for 8 min  
  
Hold:             4°C

A single isolated bacterial colony (403-42.1-P1C6-P1C3) was identified as a positive  
25 colony to contain PSKA cDNA. The plasmid and clone was given ID as pc3-  
PSKA#403-1. The plasmid DNA was prepared using midi-plasmid preparation kit  
(Qiagen, catalogue no. 12245) to use for sequencing and transfections.

**Example 2****Cloning of SKB****5 A. Screening of 120K bacterial pools and 10K bacterial**

All fifty one 120K bacterial pools were amplified under the following condition of PCR amplification by using Expand™ PCR system from Boehringer Mannheim (Catalogue no. 1681-842) with the following pair of primers.

10

PSKB-C4F2            5' TGC AAA TCT CTA GAA GAT GAC GGT G 3'

PSKB-C4R3            5' TAT ACT CAA ACT ACT GGT CTC TCC AAG 3'

Each reaction contained the following reagents:

15

2 µl of 10x PCR Buffer 3

0.4 µl of 25mM dNTP mix

0.6 µl of Primer PSKB-C4F2 (10pm/µl)

0.6 µl of Primer PSKB-C4R3 (10pm/µl)

20 

0.3 µl of Enzyme (3unit)

15.1 µl water

1 µl DNA

PCR conditions:

25 

Incubate:            94°C for 2 min

30 cycles:            94°C for 1 min

62°C for 1 min

68°C for 1 min

Incubate:            68°C for 8 min

30

Hold:                4°C

A DNA fragment of approximately 500bp was amplified from four 120K bacterial pools.

## 5 B. Screening of 10K bacterial pools

10K pools of positive three 120K pools were amplified under the following condition of PCR amplification by using Expand <sup>TM</sup> PCR system from Boehringer Mannheim (Catalogue no. 1681-842) with the following pair of primers.

10

PSKB-C4F2            5' TGC AAA TCT CTA GAA GAT GAC GGT G 3'

PSKB-C4R3            5' TAT ACT CAA ACT ACT GGT CTC TCC AAG 3'

Each reaction contained the following reagents:

15

2 µl of 10x PCR Buffer 3

0.4 µl of 25mM dNTP mix

0.6 µl of Primer PSKB-C4F2 (10pm/µl)

0.6 µl of Primer PSKB-C4R3 (10pm/µl)

20 

0.3 µl of Enzyme (3unit)

15.1 µl water

1 µl DNA

PCR conditions:

25 

Incubate:            94°C for 2 min

32 cycles:            94°C for 1 min

58°C for 40 sec

68°C for 40 sec

Incubate:            68°C for 8 min

30

Hold:                4°C

Two 10K bacterial pools (308 and 532) were found positive.

### C. Isolation of PSKB clone from 10K bacterial pool #532 by PCR Screening

5

The following three steps and three rounds of PCR were used to isolate individual positive clone of PSKB cDNA from 10K bacterial pool #532.

1. 10K bacterial pool #532 was plated on agar plates. 100-500 colonies were scraped  
10 in sub-pool and re-suspended in 100 µl of 2XYT + 20% glycerol. The bacterial re-suspensions were used as template for PCR screening.
2. The positive sub-pool of 100-500 bacterial colonies was plated on agar plates. 20-50 colonies were scraped in sub-pool and re-suspended in 100 µl of 2XYT + 20% glycerol. The bacterial re-suspensions were used as template for PCR screening.
- 15 3. The positive sub-pool of 20-50 bacterial colonies was plated on agar plates. The individual bacterial colonies were scraped and re-suspended in 100 µl of 2XYT + 20% glycerol. The bacterial re-suspensions were used as template for PCR screening.

- 20 The PCR screening was done by using Expand™ PCR system from Boehringer Mannheim (Catalogue no. 1681-842) with the following pair of primers.

PSKB-C4F2            5' TGC AAA TCT CTA GAA GAT GAC GGT G 3'

PSKB-C4R3            5' TAT ACT CAA ACT ACT GGT CTC TCC AAG 3'

25

Each reaction contained the following reagents:

2 µl of 10x PCR Buffer 3

0.4 µl of 25mM dNTP mix

- 30 0.6 µl of Primer PSKB-C4F2 (10pm/µl)

0.6 µl of Primer PSKB-C4R3 (10pm/µl)

0.3 µl of Enzyme (3unit)

15.1 µl water

1 µl DNA

5    PCR conditions:

Incubate:            94°C for 2 min

30 cycles:           94°C for 1 min

                         58°C for 40 sec

                         68°C for 40 sec

10   Incubate:           68°C for 8 min

Hold:                4°C

15   Two colonies (532 - P1A9 - P1G1 - P1E4 and 532 - P1A9 - P1G1 - P1E9) were found positive. They were given ID of pc3-PSKB#532-1 and pc3-PSKB#532-2. The plasmid DNA was prepared using mini-plasmid preparation kit (Qiagen, catalogue no. 12245) to use for sequencing and transfections.

20

**Example 3**

**Cloning of SKC**

**A. Screening of 120K bacterial pools and 10K bacterial**

25   All fifty one 120K bacterial pools were amplified under the following condition of PCR amplification by using Expand TM PCR system from Boehringer Mannheim (Catalogue no. 1681-842) with the following pair of primers.

PSKC-F2            5' TTA ACA TAG ACA AAT ACG ACG GCA TCG 3'

30   PSKC-R1           5' ACA CAT CCA TGG CCA GCG AGT CC 3'

Each reaction contained the following reagents:

- 2 µl of 10x PCR Buffer 3
- 0.4 µl of 25mM dNTP mix
- 5 0.6 µl of Primer PSKC-F2 (10pm/µl)
- 0.6 µl of Primer PSKC-R1 (10pm/µl)
- 0.3 µl of Enzyme (3unit)
- 15.1 µl water
- 1 µl DNA

10

PCR conditions:

- Incubate: 94°C for 2 min
- 30 cycles: 94°C for 40 sec
- 58°C for 40 sec
- 15 68°C for 40 sec
- Incubate: 68°C for 8 min
- Hold: 4°C

- 20 A DNA fragment of approximately 250bp was amplified from ten 120K bacterial pools.

#### B. Screening of 10K bacterial pools

- 25 10K pools of the four positive 120K pools were amplified under the following condition of PCR amplification by using Expand <sup>TM</sup> PCR system from Boehringer Mannheim (Catalogue no. 1681-842) with the following pair of primers.

- PSKC-F2 5' TTA ACA TAG ACA AAT ACG ACG GCA TCG 3'
- 30 PSKC-R1 5' ACA CAT CCA TGG CCA GCG AGT CC 3'

Each reaction contained the following reagents:

- 2 µl of 10x PCR Buffer 3
- 0.4 µl of 25mM dNTP mix
- 5 0.6 µl of Primer PSKC-F2 (10pm/µl)
- 0.6 µl of Primer PSKC-R1 (10pm/µl)
- 0.3 µl of Enzyme (3unit)
- 15.1 µl water
- 1 µl DNA

10

PCR conditions:

- Incubate: 94°C for 2 min
- 30 cycles: 94°C for 40 sec
- 58°C for 40 sec
- 15 68°C for 40 sec
- Incubate: 68°C for 8 min
- Hold: 4°C

20

Three 10K bacterial pools (64, 320 and 330) were found positive.

#### C. Isolation of PSKC clone from 10K bacterial pool #330 by PCR Screening

25

The following three steps and three rounds of PCR were used to isolate individual positive clone of PSKC cDNA from 10K bacterial pool #330.

- 4. 10K bacterial pool #330 was plated on agar plates. 100-500 colonies were scraped
- 30 in sub-pool and re-suspended in 100 µl of 2XYT + 20% glycerol. The bacterial re-suspensions were used as template for PCR screening.



5. The positive sub-pool of 100-500 bacterial colonies was plated on agar plates. 20-50 colonies were scraped in sub-pool and re-suspended in 100  $\mu$ l of 2XYT + 20% glycerol. The bacterial re-suspensions were used as template for PCR screening.
  6. The positive sub-pool of 20-50 bacterial colonies was plated on agar plates. The individual bacterial colonies were scraped and re-suspended in 100  $\mu$ l of 2XYT + 20% glycerol. The bacterial re-suspensions were used as template for PCR screening.
- 10 The PCR screening were done by using Expand TM PCR system from Boehringer Mannheim (Catalogue no. 1681-842) with the following pair of primers.

PSKC-F2                    5' TTA ACA TAG ACA AAT ACG ACG GCA TCG 3'  
 PSKC-R1                    5' ACA CAT CCA TGG CCA GCG AGT CC 3'

15

Each reaction contained the following reagents:

- 2  $\mu$ l of 10x PCR Buffer 3  
 0.4  $\mu$ l of 25mM dNTP mix  
 20 0.6  $\mu$ l of Primer PSKC-F2 (10pm/ $\mu$ l)  
 0.6  $\mu$ l of Primer PSKC-R1 (10pm/ $\mu$ l)  
 0.3  $\mu$ l of Enzyme (3unit)  
 15.1  $\mu$ l water  
 1  $\mu$ l DNA

25

PCR conditions:

- Incubate:                    94°C for 2 min  
 30 cycles:                    94°C for 40 sec  
                                   58°C for 40 sec  
 30                                68°C for 40 sec  
 Incubate:                    68°C for 8 min

Hold: 4°C

- 5 Two colonies (330 – P1G3 – P1B8 – P2A9 and 330 – P1G3 – P4E10– P1B12) were found positive. They were given ID of pc3-PSKC#330-1 and pc3-PSKC#330-2. The plasmid DNA was prepared using mini-plasmid preparation kit (Qiagen, catalogue no. 12245) to use for sequencing and transfections.

10 **Example 4                                      Phosphorylation activity of Human Sphingosine Kinase**

**A) Protocol of Phosphorylation Assay using Swiss 3T3 and 293-EBNA cells—  
Phosphate label**

15

- 1) Swiss 3T3 cells were washed with PBS and harvested by scraping in 1 ml of protein buffer [0.1 M Tris-HCl, pH 7.4 containing 20% glycerol (v/v), 1mM mercaptoethanol, 1 mM EDTA, 1 mM Na<sub>3</sub>VO<sub>4</sub> (Sigma, cat # S6508), 15 mM NaF, 10µg/ml leupeptin (Sigma, cat # L2023) and aprotinin (Sigma, cat# A6279),  
20 1 mM PMSF and 0.5 mM 4-deoxypyridoxine(Sigma, cat # D0501)] (as described in Edsall et al., 1997).
- 2) Methods were taken from Edsall et al., 1997, with the following exceptions: once cells were lysed by freeze-thawing three times, the cytosolic fraction was prepared by centrifugation at 13,000 x g for 20 min at 4°C.
- 25 3) The phosphorylation reaction included, 80 µl of cytosolic fraction with 10 µl of sphingosine (Calbiochem, cat # 219535-S) (100 µM dissolved in a 4mg/ml solution of BSA). The reactions were started by adding 10 µl of [ $\gamma$  <sup>33</sup>P]-ATP (10-20 µCi, 10 mM) (Amersham cat # AH 9968) prepared in 100 mM MgCl<sub>2</sub>. Samples were incubated at 37°C for one hour.
- 30 4) Lipids were extracted with 800 µl chloroform:methanol:concentrated HCl (100:200:1). Samples were vortexed and phases were separated by adding 240 µl

of chloroform and 240  $\mu$ l of 2M KCl (as described in Olivera et al., 1994).

Samples were vortexed and centrifuged at 11,000 x g for 5 min.

- 5) Lipids found in the organic phase were spotted on a TLC silica gel plate and run in the following solvent system: chloroform:methanol:acetic acid:water (60:30:5:5). A sphingosine 1-phosphate (S 1-P) (Sigma, cat #S-9666) and sphingosine (Sph) (standard was run alongside all experimental samples as well as a reaction tube containing no sphingosine as a negative control.
- 6) The sphingosine 1-phosphate and sphingosine standards were visualized using a  $\text{KMnO}_4$  stain (100 ml  $\text{H}_2\text{O}$ : 4g  $\text{KMnO}_4$ , 4g  $\text{NaHCO}_3$ ). TLC plates were exposed to a phosphor screen overnight.
- 7) S 1-P bands were visualized and quantified with the Storm Phosphoimager (Molecular Dynamics, Sunnyvale, CA).

#### B) Transient transfection protocol for 293-EBNA

Day 1.

- 1) 100 mm plates of 293-EBNA with a confluency of 50-80% were used for transfection.
- 2) SKA, SKB, SKC and pcDNA3 (4  $\mu$ g) DNA samples were diluted in 750  $\mu$ l of DMEM/F12 (serum-free media) and 20  $\mu$ l Plus Reagent (Lipofectamine Plus Kit, Life Technologies Cat. 10964-013), and incubated at room temperature for 15 min.
- 3) 30  $\mu$ l Lipofectamine Reagent (Lipofectamine Plus Kit) was diluted in 750  $\mu$ l DMEM/F12. The diluted Lipofectamine was then combined with the DNA/Plus mixture and incubated at RT for 15 min.
- 4) The 293-EBNA plates were washed once with PBS, and 5 ml DMEM/F12 was added to each plate.
- 5) DNA/Plus/Lipofectamine mixture was added to each plate of 293-EBNA cells. The plates were left for 3 hr at 37°C in a 5%  $\text{CO}_2$  incubator.
- 6) The transfection medium was replaced with DMEM/F12 containing 10% FBS to recover overnight.

Day 3.

Media was removed and stored at  $-80^{\circ}\text{C}$ . Cells were washed once with PBS and were harvested by scrapping cells in 1 ml of protein buffer (described above). Cells were lysed by freeze-thawing three times. Cytosolic fractions were obtained by centrifugation at  $13,000 \times g$  for 20 min at  $4^{\circ}\text{C}$ . Pellets of cell debris resulting from this spin was stored at  $-80^{\circ}\text{C}$  for later use. Cytosolic protein preparations were stored at  $-80^{\circ}\text{C}$  for future use.

10 C) Phosphorylation Assay using Three Fractions of SKA, SKB and SKC.

i) *Cytosolic Fraction.*

The phosphorylation assay was performed as outlined above, except 5  $\mu\text{l}$  of Triton X-100 was added to each reaction tube.

15 ii) *Debris Fraction*

Each pellet consisting of cellular debris was re-suspended in 80  $\mu\text{l}$  of protein buffer. The suspension was sonicated. This prep was used for the phosphorylation assay which was as outlined above in the cytosolic fraction.

iii) *Cell Media*

20 The cell media was dried down in a refrigerated speed-vac. The pellet was re-suspended in 80  $\mu\text{l}$  of protein buffer. This preparation was used for the phosphorylation assay which was as outlined above in the cytosolic fraction.

25 D) Protocol of Phosphorylation Assay using 293-EBNA cells—Compound Label

Enzyme preparations of SKA and pcDNA3 were used in phosphorylation assays.

The protocol was the same as mentioned above in A.3-7 with a few exceptions:

i) 5  $\mu\text{l}$  of Triton X-100 was added to each reaction tube and ii)  $^{33}\text{P}$ -ATP was not used, only "cold" ATP was used in each reaction (10 mM in 100 mM  $\text{MgCl}_2$ ).

30

The results for the above phosphorylation assays indicated that SKA was involved in phosphorylating sphingosine to form sphingosine 1-phosphate. In particular, as indicated in Figure 11, SKA was shown to be involved in phosphorylating sphingosine whereas the tests did not exemplify phosphorylation by the cloned SKB and SKC genes.

#### **Example 5                      Antisense analysis**

Knowledge of the correct, complete cDNA sequence of human SK enables its use as a tool for antisense technology in the investigation of gene function. Oligonucleotides, cDNA or genomic fragments comprising the antisense strand of SK are used either in vitro or in vivo to inhibit expression of the mRNA. Such technology is now well known in the art, and antisense molecules can be designed at various locations along the nucleotide sequences. By treatment of cells or whole test animals with such antisense sequences, the gene of interest is effectively turned off. Frequently, the function of the gene is ascertained by observing behavior at the intracellular, cellular, tissue or organismal level (e.g., lethality, loss of differentiated function, changes in morphology, etc.).

In addition to using sequences constructed to interrupt transcription of a particular open reading frame, modifications of gene expression is obtained by designing antisense sequences to intron regions, promoter/enhancer elements; or even to trans-acting regulatory genes. Similarly, inhibition is achieved using Hogeboom base-pairing methodology, also known as "triple helix" base pairing.

#### **Example 6                      Expression of Human SK**

Expression of human SK is accomplished by subcloning the cDNAs into appropriate expression vectors and transfecting the vectors into analogous expression hosts for example E.Coli. In a particular case, the vector is engineered such that it contains a promoter for  $\beta$ -galactosidase, upstream of the cloning site, followed by

sequence containing the amino-terminal Met and the subsequent 7 residues of  $\beta$ -galactosidase. Immediately following these eight residues is an engineered bacteriophage promoter useful for artificial priming and transcription and for providing a number of unique endonuclease restriction sites for cloning.

5

Induction of the isolated, transfected bacterial strain with IPTG using standard methods produces a fusion protein corresponding to the first seven residues of  $\beta$ -galactosidase, about 15 residues of "linker", and the peptide encoded within the cDNA. Since cDNA clone inserts are generated by an essentially random process, there is one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, it is obtained by deletion or insertion of the appropriate number of bases using well known methods including in vitro mutagenesis, digestion with exonuclease III or mung bean nuclease, or the inclusion of an oligonucleotide linker of appropriate length.

15

The human SK cDNA is shuttled into other vectors known to be useful for expression of protein in specific hosts. Oligonucleotide primers containing cloning sites as well as a segment of DNA (about 25 bases) sufficient to hybridize to stretches at both ends of the target cDNA is synthesized chemically by standard methods. These primers are then used to amplify the desired gene segment by PCR. The resulting gene segment is digested with appropriate restriction enzymes under standard conditions and isolated by gel electrophoresis. Alternately, similar gene segments are produced by digestion of the cDNA with appropriate restriction enzymes. Using appropriate primers, segments of coding sequence from more than one gene are ligated together and cloned in appropriate vectors. It is possible to optimize expression by construction of such chimeric sequences.

20  
25

Suitable expression hosts for such chimeric molecules include, but are not limited to, mammalian cells such as Chinese Hamster Ovary (CHO) and human 293 cells, insect cells such as Sf9 cells, yeast cells such as *Saccharomyces cerevisiae*, and bacteria such as *E. coli*. For each of these cell systems, a useful expression vector

30

also includes an origin of replication to allow propagation in bacteria and a selectable marker such as the  $\beta$ -lactamase antibiotic resistance gene to allow plasmid selection in bacteria. In addition, the vector may include a second selectable marker such as the neomycin phosphotransferase gene to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts require RNA processing elements such as 3' polyadenylation sequences if such are not part of the cDNA of interest.

Additionally, the vector contains promoters or enhancers which increase gene expression. Such promoters are host specific and include MMTV, SV40, and metallothionein promoters for CHO cells; trp, lac, tac and T7 promoters for bacterial hosts; and alpha factor, alcohol oxidase and PGH promoters for yeast. Transcription enhancers, such as the rous sarcoma virus enhancer, are used in mammalian host cells. Once homogeneous cultures of recombinant cells are obtained through standard culture methods, large quantities of recombinantly produced human SK are recovered from the conditioned medium and analyzed using chromatographic methods known in the art. For example, human SK can be expressibly cloned into the expression vector pcDNA3. This product can be used to transform, for example, HEK293 or COS by methodology standard in the art. Specifically, for example, using Lipofectamine (Gibco BRL catalog no. 18324-020) mediated gene transfer.

#### Example 7

#### Isolation of Recombinant SK

Human SK is expressed as a chimeric protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (Invitrogen) between the purification domain and the human SK

sequence is useful to facilitate expression of human SK.

#### Example 8

#### Production of SK Specific Antibodies

5 Two approaches are utilized to raise antibodies to human SK, and each approach is useful for generating either polyclonal or monoclonal antibodies. In one approach, denatured protein from reverse phase HPLC separation is obtained in quantities up to 75 mg. This denatured protein is used to immunize mice or rabbits using standard protocols; about 100 micrograms are adequate for immunization of a  
10 mouse, while up to 1 mg might be used to immunize a rabbit. For identifying mouse hybridomas, the denatured protein is radioiodinated and used to screen potential murine B-cell hybridomas for those which produce antibody. This procedure requires only small quantities of protein, such that 20 mg is sufficient for labeling and screening of several thousand clones.

15

In the second approach, the amino acid sequence of an appropriate human SK domain, as deduced from translation of the cDNA, is analyzed to determine regions of high antigenicity. Oligopeptides comprising appropriate hydrophilic regions are synthesized and used in suitable immunization protocols to raise antibodies. Analysis  
20 to select appropriate epitopes is described by Ausubel FM et al (supra). The optimal amino acid sequences for immunization are usually at the C-terminus, the N-terminus and those intervening, hydrophilic regions of the polypeptide which are likely to be exposed to the external environment when the protein is in its natural conformation.

25

Typically, selected peptides, about 15 residues in length, are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to keyhole limpet hemocyanin (KLH; Sigma, St. Louis MO) by reaction with M-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel FM et al, supra). If necessary, a cysteine is introduced at the N-terminus of the peptide to permit  
30 coupling to KLH. Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding



the peptide to plastic, blocking with 1% bovine serum albumin, reacting with antisera, washing and reacting with labeled (radioactive or fluorescent), affinity purified, specific goat anti-rabbit IgG.

Hybridomas are prepared and screened using standard techniques. Hybridomas of interest are detected by screening with labeled human SK to identify those fusions producing the monoclonal antibody with the desired specificity. In a typical protocol, wells of plates (FAST; Becton-Dickinson, Palo Alto CA) are coated during incubation with affinity purified, specific rabbit anti-mouse (or suitable antispecies Ig) antibodies at 10 mg/ml. The coated wells are blocked with 1% BSA, washed and incubated with supernatants from hybridomas. After washing the wells are incubated with labeled human SK at 1 mg/ml. Supernatants with specific antibodies bind more labeled human SK than is detectable in the background. Then clones producing specific antibodies are expanded and subjected to two cycles of cloning at limiting dilution. Cloned hybridomas are injected into pristane-treated mice to produce ascites, and monoclonal antibody is purified from mouse ascetic fluid by affinity chromatography on Protein A. Monoclonal antibodies with affinities of at least  $10^8$  M<sup>-1</sup>, preferably  $10^9$  to  $10^{10}$  or stronger, are typically made by standard procedures as described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; and in Goding (1986) Monoclonal Antibodies: Principles and Practice, Academic Press, New York City, both incorporated herein by reference.

25 **Example 9**                      **Diagnostic Test Using Human SK Specific**  
**Antibodies**

Particular Human SK antibodies are useful for investigating signal transduction and the diagnosis of infectious or hereditary conditions which are characterized by differences in the amount or distribution of human SK or downstream products of an active signaling cascade.

Diagnostic tests for human SK include methods utilizing antibody and a label to detect human SK in human body fluids, membranes, cells, tissues or extracts of such. The polypeptides and antibodies of the present invention are used with or without modification. Frequently, the polypeptides and antibodies are labeled by joining them, either covalently or noncovalently, with a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and have been reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, chromogenic agents, magnetic particles and the like. Patents teaching the use of such labels include US Patent No's. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in US Patent No.4,816,567, Incorporated herein by reference.

A variety of protocols for measuring soluble or membrane-bound human SK, using either polyclonal or monoclonal antibodies specific for the protein, are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on human SK is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, DE et al (1983, J Exp. Med. 158:1211f).

#### **Example 10                      Purification of Native Human SK Using Specific Antibodies**

Native or recombinant human SK is purified by immunoaffinity chromatography using antibodies specific for human SK. In general, an immunoaffinity column is constructed by covalently coupling the anti-TRH antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway NJ). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or  
5 chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated Sepharose (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

10

Such immunoaffinity columns are utilized in the purification of human SK by preparing a fraction from cells containing human SK in a soluble form. This preparation is derived by solubilization of whole cells or of a subcellular fraction obtained via differential centrifugation (with or without addition of detergent) or by  
15 other methods well known in the art. Alternatively, soluble human SK containing a signal sequence is secreted in useful quantity into the medium in which the cells are grown.

#### Example 11

#### Drug Screening

20

This invention is particularly useful for screening therapeutic compounds by using human SK or binding fragments thereof in any of a variety of drug screening techniques. For example, human SK activity can be measured using any of a variety of appropriate functional assays in which activation of the kinase results in an  
25 observable change in the level of a particular product. Thus, the present invention provides methods of screening for drugs or any other agents which are affected by human SK.

Alternatively, the polypeptide or fragment employed in such a test is either free in solution, affixed to a solid support, borne on a cell surface or located  
30 intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells (or membrane preparations therefrom) which are stably transformed with

recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells. One measures, for example, the formation of a phosphorylated product and compares that with a control.

## 5      **Example 12**                              **Rational Drug Design**

Herein, the goal of rational drug design is to produce structural analogs of biologically active lipids of interest or of small molecules with which they interact, agonists, antagonists, or inhibitors.

10

In one approach, the three-dimensional structure of a protein of interest, or of a protein-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to  
15 determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide is gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design efficient inhibitors. Useful examples of rational drug design includes molecules which have improved activity or stability as shown by Braxton S and Wells JA (1992, Biochemistry 31:7796-7801) or which act as inhibitors, agonists, or antagonists of  
20 native peptides as shown by Athauda SB et al (1993 J Biochem 113:742-46), incorporated herein by reference.

## 25      **Example 13**                              **Use and Administration of Antibodies, Inhibitors, or Antagonists**

Antibodies, inhibitors, or antagonists of human SK (or other treatments to limit signal transduction, LST) provide different effects when administered therapeutically. LSTs are formulated in a nontoxic, inert, pharmaceutically  
30 acceptable aqueous carrier medium preferably at a pH of about 5 to 8, more preferably 6 to 8, although pH may vary according to the characteristics of the

antibody, inhibitor, or antagonist being formulated and the condition to be treated. Characteristics of LSTs include solubility of the molecule, half-life and antigenicity/immunogenicity. These and other characteristics aid in defining an effective carrier.

5

LSTs are delivered by known routes of administration including but not limited to topical creams and gels; transmucosal spray and aerosol; transdermal patch and bandage; injectable, intravenous and lavage formulations; and orally administered liquids and pills particularly formulated to resist stomach acid and enzymes. The particular formulation, exact dosage, and route of administration is determined by the attending physician and varies according to each specific situation.

Such determinations are made by considering multiple variables such as the condition to be treated, the LST to be administered, and the pharmacokinetic profile of a particular LST. Additional factors which are taken into account include severity of the disease state, patient's age, weight, gender and diet, time and frequency of LST administration, possible combination with other drugs, reaction sensitivities, and tolerance/response to therapy. Long acting LST formulations might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular LST.

Normal dosage amounts vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see US Patent Nos. 4,657,760; 5,206,344; or 5,225,212. Those skilled in the art employ different formulations for different LSTs. Administration to cells such as nerve cells necessitates delivery in a manner different from that to other cells such as vascular endothelial cells.

It is contemplated that abnormal signal transduction, trauma, or diseases which trigger humans SK activity are treatable with LSTs. These conditions or

diseases are specifically diagnosed by the tests discussed above, and such testing should be performed in suspected cases of viral, bacterial or fungal infections; allergic responses; mechanical injury associated with trauma; hereditary diseases; lymphoma or carcinoma; or other conditions which activate the genes of lymphoid or neuronal tissues.

All publications and patent applications mentioned herein are incorporated by reference for the purpose of describing the methodologies, cell lines and vectors, among other things. However, nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure, for example, by virtue of prior invention.

Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments.

**Claims**

1. An isolated polynucleotide comprising a sequence encoding human sphingosine kinase as selected from the group consisting of:
  - 5 (a) human sphingosine kinase A or variants thereof;
  - (b) human sphingosine kinase B or variants thereof; and
  - (c) human sphingosine kinase C or variants thereof.
2. The isolated polynucleotide of claim 1 wherein said sequence encodes human  
10 sphingosine kinase A or variants thereof.
3. The isolated polynucleotide of claim 2 wherein said sequence encodes the sequence of Figure 3.
- 15 4. The isolated polynucleotide of claim 1 wherein said sequence encodes human sphingosine kinase B or variants thereof.
5. The isolated polynucleotide of claim 4 wherein said sequence encodes the sequence of Figure 6.  
20
6. The isolated polynucleotide of claim 1 wherein said sequence encodes human sphingosine kinase C or variants thereof.
7. The isolated polynucleotide of claim 6 wherein said sequence encodes the  
25 sequence of Figure 9.
8. An isolated polynucleotide sequence comprising a complement of claim 1.
9. A composition containing the isolated polynucleotide sequence of claim 8 and an  
30 acceptable excipient.

10. An expression construct containing the isolated polynucleotide of claim 1.
11. A host cell containing the expression vector of claim 10.
- 5 12. A method for making a purified polypeptide comprising the amino acid sequence for human sphingosine kinase wherein the method comprises the steps of culturing a host cell of claim 11 in suitable conditions to express said polypeptide and isolating and purifying said expressed polypeptide.
- 10 13. A purified polypeptide comprising the amino acid sequence for human sphingosine kinase prepared by the method of claim 12.
14. A method of screening a compound for determining the capability of said compound to inhibit or activate human sphingosine kinase activity, which method  
15 comprises:
  - contacting a host cell of claim 11 with said compound; and
  - measuring the inhibition or activation of human sphingosine kinase activity.



Figure 1 Full-length human PSKA cDNA.

GAGGTTATGGATCCAGCGGGCGGGCCCCCGGGGCGTGCTCCCGCGGCCCTGCCGCGTGCTG  
1 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 60  
CTCCAATACCTAGGTCTGCCCCGCGGGGGCCCCGCACGAGGGCGCCGGGACGGCGCACGAC  
GTGCTGCTGAACCCGCGCGGGCGGCAAGGGCAAGGCCTTGCAGCTCTTCCGGAGTCAAGTG  
61 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 120  
CACGACGACTTGGGCGCGCCCGTTCCTCGTTCGGAACGTCGAGAAGGCCTCAGTGCAC  
CAGCCCCCTTTGGCTGAGGCTGAAATCTCCTTCACGCTGATGCTCACTGAGCGGCGGAAC  
121 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 180  
GTCGGGGAAAACCGACTCCGACTTTAGAGGAAGTGCAGCTACGAGTGACTCGCCGCTTG  
CACGCGCGGGAGCTGGTGCGGTCTGGAGGAGCTGGGCGGCTGGGACGCTCTGGTGCTCATG  
181 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 240  
GTGCGCGCCCTCGACCAGCCAGCCTCCTCGACCCGGCGACCCTGCGAGACCACCACTAC  
TCTGGAGACGGGCTGATGCACGAGGTGGTGAACGGGCTCATGGAGCGGCTGACTGGGAG  
241 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 300  
AGACCTCTGCCCCACTACGTGCTCCACCCTTGCCCGAGTACCTCGCCGACTGACCCCTC  
ACCGCCATCCAGAAaCCCCTGTGTATCCTCCAGCAGGCTCTGGCAACGCGCTGGCAGCT  
301 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 360  
TGGCGGTAGGTCTTtGGGGACACATAGGAGGGTCTGTCGAGACCGTTGCGCGACCGTCTGA  
TCCTTGAACCATTATGCTGGCTATGAGCAGGTACCAATGAAGACCTCCTGACCAACTGC  
361 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 420  
AGGAACTTGTAATACGACCGATACTCGTCCAGTGGTTACTTCTGGAGGACTGGTTGACG  
ACGCTATTGCTGTGCGCCGGCTGCTGTCAACCATGAACCTGCTGTCTCTGCACACGGCT  
421 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 480  
TGCGATAACGACACGGCGGCGGACGACAGTGGGTAAGTGGACGACAGAGACGTGTGCCGA  
TCGGGGCTGCGCCTCTTCTCTGTGCTCAGCCTGGCCTGGGGCTTCATTGCTGATGTGGAC  
481 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 540  
AGCCCCGACGCGGAGAAGAGACACGAGTCTGACCGGACCCCGAAGTAACGACTACACCTG  
CTAGAGAGTGAGAAGTATCGGCGTCTGGGGGAGATGCGCTTCACTCTGGGCACCTTCCTG  
541 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 600  
GATCTCTCACTCTTCATAGCCGAGACCCCTCTACGCGAAGTGAGACCCGTGGAAGGAC  
CGTCTGGCAGCCCTGCGCACCTACCGCGGCGGACTGGCCTACCTCCCTGTAGGAAGAGTG  
601 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 660  
GCAGACCGTCTGGGACGCGTGGATGGCGCCGCTGACCGGATGGAGGGACATCCTTCTCAC  
GGTTCCAAGACACCTGCCTCCCCCGTTGTGGTCCAGCAGGGCCCCGGTAGATGCACACCTT  
661 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 720  
CCAAGGTTCTGTGGACGGAGGGGGCAACACAGGTCGTCCCGGGCCATCTACGTGTGGAA  
GTGCCACTGGAGGAGCCAGTGCCCTCTCACTGGACAGTGGTGCCCGACGAGGACTTTGTG  
721 -----+-----+-----+-----+-----+-----+-----+-----+-----+ 780  
CACGGTGACCTCCTCGGTACGGGAGAGTGACCTGTCACCACGGGCTGCTCCTGAAACAC

2/22

Figure 1 cont'd

781 CTAGTCCTGGCACTGCTGCACTCGCACCTGGGCAGTGAGATGTTTGCTGCACCCATGGGC  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
GATCAGGACCGTGACGACGTGAGCGTGGACCCGTCCTCTACAAACGACGTGGGTACCCG 840  
CGCTGTGCAGCTGGCGTCAATGCATCTGTTCTACGTGCGGGCGGGAGTGTCTCGTGCCATG  
841 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 900  
GCGACACGTGACCGCAGTACGTAGACAAGATGCACGCCCGCCCTCACAGACACGGTAC  
CTGCTGCGCCTCTTCTGCGCATGGAGAAGGGCAGGCATATGGAGTATGAATGCCCCCTAC  
901 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 960  
GACGACGCGGAGAAGGACCGGTACCTCTTCCCGTCCGTATACCTCATACTTACGGGGATG  
TTGGTATATGTGCCCCGTGGTCGCCTTCCGCTTGGAGCCCAAGGATGGGAAAGGTGTGTTT  
961 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1020  
AACCATATACACGGGCACCAGCGGAAGGCGAACCTCGGGTTCCTACCCTTTCCACACAAA  
GCAGTGGATGGGGAATTGATGGTTAGCGAGCCGTGCAGGGCCAGGTGCACCCAACTAC  
1021 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1080  
CGTCACCTACCCCTTAACCTACCAATCGCTCGGCACGTCCCGGTCCACGTGGGTTTGATG  
TTCTGGATGGTCAGCGGTTGCGTGGAGCCCCCGCCAGCTGGAAGCCCCAGCAGATGCCA  
1081 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1140  
AAGACCTACCACTCGCCAACGCACCTCGGGGGCGGGTTCGACCTTCGGGGTCGTCTACGGT  
CCGCCAGAAGAGCCCTTATGACCCCTGGGCGCGCTGTGCCTTAGTGTCTACTTGCAGGA  
1141 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1200  
GGCGGTCTTCTCGGGAATACTGGGGACCCGGCGGACACGGAATCACAGATGAACGTCT  
CCCTTCCTCCTTCCCTAGGGCTGCAGGGCCTGTCCACAGCTCCTGTGGGGGTGGAGGAGA  
1201 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1260  
GGGAAGGAGGAAGGGATCCCGACGTCCCGGACAGGTGTCGAGGACACCCCCACCTCCTCT  
CTCCTCTGGAGAAGGGTGAGAAGGTGGAGGCTATGCTTTGGGGGGACAGGCCAGAATGAA  
1261 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1320  
GAGGAGACCTCTTCCCACTCTTCCACCTCCGATACGAAACCCCCCTGTCCGGTCTTACTT  
GTCCTGGGTGAGGAGCCCAGCTGGCTGGGCCCAGCTGCCTATGTAAGGCCTTCTAGTTTG  
1321 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1380  
CAGGACCCAGTCCTCGGGTCGACCGACCCGGGTGACGGATACATTCCGGAAGATCAAAC  
TTCTGAGACCCCCACCCACGAACCAAATCCAAATAAAGTGACATTCACAAAAA  
1381 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1440  
AAGACTCTGGGGGTGGGGTGCTTGGTTTAGGTTTATTTCACTGTAAGGGTTTTTTTTTT  
AAAAAAA  
1441 ----- 1447  
TTTTTTT



4/22

Figure 2 cont'd

```
847  -----+-----+-----+-----+-----+-----+-----+-----+-----+----- 906
      CGTCGACCGCAGTACGTAGACAAGATGCACGCCCGCCCTCACAGAGCACGGTACGACGAC
      CGCCTCTTCCTGGCCATGGAGAAGGGCAGGCATATGGAGTATGAATGCCCCCTACTTGGTA
907  -----+-----+-----+-----+-----+-----+-----+-----+-----+----- 966
      GCGGAGAAGGACCGGTACCTCTTCCCGTCCGTATACCTCATACTTACGGGGATGAACCAT
      TATGTGCCCCGTGGTCGCCTTCCGCTTGGAGCCCAAGGATGGGAAAGGTGTGTTTGCAGTG
967  -----+-----+-----+-----+-----+-----+-----+-----+-----+----- 1026
      ATACACGGGCACCAGCGGAAGGCGAACCTCGGGTTCCTACCCTTTCCACACAAACGTCAC
      GATGGGGAATTGATGGTTAGCGAGCCGTGCAGGGCCAGGTGCACCCAACTACTTCTGG
1027 -----+-----+-----+-----+-----+-----+-----+-----+-----+----- 1086
      CTACCCCTTAACTACCAATCcgCTCGGCACGTCCCGGTCCACGTGGGTTTGATGAAGACC
      ATGGTCAGCGGTTGCGTGGAGCCCCCGCCAGCTGGAAGCCCCAGCAGATGCCACCGCCA
1087 -----+-----+-----+-----+-----+-----+-----+-----+-----+----- 1146
      TACCAGTCGCCAACGCACCTCGGGGGCGGGTCGACCTTCGGGGTCGTCTACGGTGGCGGT
      GAAGAGCCCTTATGA
1147 -----+-----+-----+-----+-----+-----+-----+-----+-----+----- 1161
      CTTCTCGGGAATACT
```

5/22

Figure 3. Predicted amino acid sequence of human PSKA protein.

```
1  MDPAGGPRGVLPRPCRVLVLLNPRGGKGKALQLFRSHVQPLLAEAEISFTLMLTERRNHA 60
   -----+-----+-----+-----+-----+-----+-----+
61  RELVRSEELGRWDALVVMGSDGLMHEVVNGLMERPDWETAIQKPLCILPAGSGNALAASL 120
   -----+-----+-----+-----+-----+-----+-----+
121 NHYAGYEQVTNEDLLTNCTLLLCRRLLSPMNLSSLHTASGLRFLSVLSLAWGFIADVDLE 180
   -----+-----+-----+-----+-----+-----+-----+
181 SEKYRRLGEMRFTLGTFLRLAALRTYRGLAYLPVGRVGSKTPASPVVQQGPVDAHLVP 240
   -----+-----+-----+-----+-----+-----+-----+
241 LEEPVP SHWTVVPDEDFVLVLALLHSHLGSEMFAAPMGRCAAGVMHLFYVRAGVSRAMLL 300
   -----+-----+-----+-----+-----+-----+-----+
301 RLFLAMEKGRHMEYECPLYVYPVVAFRLEPKDGKGVFAVDGELMVRRAVQGQVHPNYFW 360
   -----+-----+-----+-----+-----+-----+-----+
361 MVSGCVEPPPSWKPPQMPPPEEPL* 385
   -----+-----+-----+-----+-----+-----+-----+
```

6/22

Figure 4. Sequence of full-length cDNA encoding human PSKB.

AGCCGCGAGCTGGACCAGCCGTGCAAATCTCTAGAAGATGACGGTGTCTTTAAAACGCT  
1 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 60  
TCGSCGCTCGACCTGGTCGGCACGTTTAGAGATCTTCTACTGCCACAAGAAATTTGCGA  
TCGAAATCACTGGAAGAAAACCTACAGCTGGGCTCTGCCTGCTGACCTGGGGAGGCCATTG  
61 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 120  
AGCTTTAGTGACCTTCTTTTGATGTGACCCGAGACGGACGACTGGACCCCTCCGGTAAC  
GCTCTATGGAACACTGTGATAACCTCCTAAGGAGAGCAGCCTGTCAAGAAGCTCAGGT  
121 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 180  
CGAGATACCTTTTGTGACACTATTGGAGGATTCCTCTCGTCGGACAGTTCTTCGAGTCCA  
GTTTGGCAATCAACTCATTCTCCCAATGCACAAGTGAAGAAGGCCACTGTTTTCTCAA  
181 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 240  
CAAACCGTTAGTTGAGTAAGGAGGGTTACGTGTTCACTTCTTCCGGTGACAAAAAGAGTT  
TCCTGCAGCTTGCAAAGGAAAAGCCAGGACTCTATTTGAAAAAATGCTGCCCCGATTTT  
241 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 300  
AGGACGTCGAACGTTTCCTTTTCGGTCCTGAGATAAACTTTTTTTACGACGGGGCTAAAA  
ACATTTATCTGGCATGGATGTGACTATTGTTAAGACAGATTATGAGGGACAAGCCAAGAA  
301 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 360  
TGTAATAGACCGTACCTACACTGATAACAATTCTGTCTAATACTCCCTGTTCCGTTCTT  
ACTCCTGGAACGTGATGGAAAACACGGATGTGATCATTGTTGCAGGAGGAGATGGGACACT  
361 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 420  
TGAGGACCTTGACTACCTTTTGTGCCTACACTAGTAACAACGTCCTCCTCTACCCTGTGA  
GCAGGAGGTTGTTACTGGTGTCTTCGACGAACAGATGAGGCTACCTTCAGTAAGATTCC  
421 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 480  
CGTCCTCCAACAATGACCACAAGAAGCTGCTTGTCTACTCCGATGGAAGTCATTCTAAGG  
CATTGGATTATCCCACTGGGAGAGACCAGTAGTTTGAGTCATACCCTCTTTGCCGAAAG  
481 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 540  
GTAACCTAAATAGGGTGACCCTCTCTGGTCACTCAAACTCAGTATGGGAGAAACGGCTTTC  
TGGAACAAAGTCAACATATTACTGATGCCACACTTGCCATTGTGAAAGGAGAGACAGT  
541 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 600  
ACCTTTGTTTCAGGTTGTATAATGACTACGGTGTGAACGGTAACACTTTCCTCTCTGTCA  
TCCACTTGATTTCTTGAGATCAAGGGTGAAAAGGAACAGCCTGTATTTGCAATGACCGG  
601 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 660  
AGGTGAACTAAAGAACGTCTAGTTCCCACTTTTCCTTGTCGGACATAAACGTTACTGGCC  
CCTTCGATGGGGATCTTTCAGAGATGCTGGCGTCAAAGTTAGCAAGTACTGGTATCTTGG  
661 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 720  
GGAAGCTACCCCTAGAAAGTCTCTACGACCGCAGTTTCAATCGTTCATGACCATAGAACC  
GCCTCTAAAAATCAAAGCAGCCCACTTTTTCAGCACTCTTAAGGAGTGGCCTCAGACTCA  
721 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 780  
CGGAGATTTTAGTTTCGTGGGTGAAAAAGTCGTGAGAATTCCTCACCAGGAGTCTGAGT  
TCAAGCCTCTATCTCATACACGGGACCTACAGAGAGACCTCCCAATGAACCAGAGGAGAC  
781 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 840  
AGTTCGGAGATAGAGTATGTGCCCTGGATGTCTCTCTGGAGGGTTACTTGGTCTCCTCTG  
CCCTGTACAAAGGCCTTCTTTGTACAGGAGAATATTACGAAGGCTTGCGTCCTACTGGGC

Figure 4 cont'd

7/22

841 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 900  
GGGACATGTTTCCGGAAGAAACATGTCTCTTATAATGCTTCCGAACGCAGGATGACCCG  
ACAACCACAGGATGCCCTTTCCCAAGAGGTGAGCCCGGAGGTCTGGAAAGATGTGCAGCT  
901 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 960  
TGTTGGTGTCTTACGGGAAGGGTTCTCCACTCGGGCCTCCAGACCTTTCTACACGTCTGA  
GTCCACCATTGAACTGTCCATCACAACACGGAATAATCAGCTTGACCCGACAAGCAAAGA  
961 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1020  
CAGGTGGTAACTTGACAGGTAGTGTGTGCCTTATTAGTCGAACTGGGCTGTTCTGTTTCT  
AGATTTTCTGAATATCTGCATTGAACCTGACACCATCAGCAAAGGAGACTTTATAACTAT  
1021 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1080  
TCTAAAAGACTTATAGACGTAACCTTGGACTGTGGTAGTCGTTTCTCTGAAATATTGATA  
AGGAAGTCGAAAGGTGAGAAACCCCAAGCTGCACGTGGAGGGCACGGAGTGTCTCCAAGC  
1081 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1140  
TCCTTCAGCTTTCCACTCTTTGGGGTTCGACGTGCACCTCCCGTGCCTCACAGAGGTTCC  
CAGCCAGTGCACCTTTGCTTATCCCGGAGGGAGCAGGGGGCTCTTTTAGCATTGACAGTGA  
1141 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1200  
GTCGGTCACGTGAAACGAATAGGGCCTCCCTCGTCCCCCGAGAAAATCGTAACTGTCACT  
GGAGTATGAAGCGATGCTGTGGAGGTGAACTGCTCCCCAGGAAGCTGCAGTTCTTCTG  
1201 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1260  
CCTCATACTTCGCTACGGACACCTCCACTTTGACGAGGGGTCTTCGACGTCAAGAAGAC  
TGATCCTAGGAAGAGAGAACAGATGCTCACAAGCCCCACCCAGTGAGCAGCAGAAGACAA  
1261 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1320  
ACTAGGATCCTTCTCTCTTGTCTACGAGTGTTTCGGGGTGGGTCACTCGTCGTCTTCTGTT  
GCACTCTGAGACCACACTTTAGGCCACCGGTGGGACCAAAGGGAACAGGTGCCTCAGCC  
1321 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1380  
CGTGAGACTCTGGTGTGAAATCCGGTGGCCACCCTGGTTTTCCCTTGTCCACGGAGTCGG  
ATCCCAACAGTGTCTGTCAGAGGGTCCCCAGGGCATTTCATGGCAAGTACCCCTCTGCCC  
1381 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1440  
TAGGGTTGTACAGCAGTCTCCAGGGGTCCCGTAAAAGTACCGTTTCATGGGGAGACGGG  
CCACTCCAGCAGTGCTTCCCAAAGTGTGCTCTGTACCTGCTTTGCAATCGGCTTCCATT  
1441 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1500  
GGTGAGGTCTGTCACGAAGGGTTTCACACGAGACAGTGGACGAAACGTTAGCCGAAGGTAA  
AGCGCATGTTTTATTTTGGTGTGACGGTTGGCCCTCCTAAACACGGACTTTCTCTCAGGCT  
1501 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1560  
TCGCGTACAAAATAAAACCACACTGCCAACGGGAGGATTGTGCCTGAAAGGAGTCCGA  
GGTTCAAGACGGAAAAGGACTTTCTTCTGTTTTCTTCCAAAGTGCAACCACAGTGGAGAG  
1561 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1620  
CCAAGTTCTGCCTTTCTCTGAAAGAAGACAAAAGAAGGTTTCACGTTGGTGTACCTCTC  
CCCACGGTGGGCTTAGCCTGCCTAGGCCCTTCCATTTCTTCTTTGACCGTGTCTAGGAA  
1621 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1680  
GGGTGCCACCCGAATCGGACGGATCCGGGAAGGTAAAGAGAAGAACTGGCAGCATCCTT  
TTCCAGGAAAGTGCAATTCCTGCCCTGGTGACCTTTTCTCTATGTCTAGGCTCCTCCACAGG  
1681 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1740  
AAGGTCCTTTACGTAAGGACGGGACCACTGGAAGGATAACAGATCCGAGGAGGTGTCC  
TGCTGCTATTTTGTGAGCTCCGGCTCCTGTTTAGCTTTTATTTTCAGTTCTAACCTCAGTC

Figure 4 cont'd

8/22

1741 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
ACGACGATAAAACACTCGAGGCCGAGGACAAATCGAAAATAAAGTCAAGATTGGAGTCAG 1800  
CAGAAACATATGTGAGGTTGTTTCCCTCTTCAGCCACGGCTACAATACCGGAAAATGCTA  
1801 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
GTCTTTGTATACACTCCAACAAAGGGAGAAGTCGGTGCCGATGTTATGGCCTTTTACGAT 1860  
GTTTTTATTTATTTTTTTAAGTAGTGCTTCCTAAATGGTTTGCATGAGAGCCACCTGGGG  
1861 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
CAAAAATAAATAAAAAAATTCATCACGAAGGATTTACCAAACGTACTCTCGGTGGACCCC 1920  
TACATGTTGAAAACCTATTTTGGGGTCTACCCCAAACCTAATAACCCAAATTTGGGGATGG  
1921 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
ATGTACAACCTTTGAATAAACCCAGATGGGGTTTGGATTATTGGGTTTTAAACCCCTACC 1980  
GGCCCAGGAATATGCATTTTTTAAAAAGTCATCTGCCCTTCCCAGGTGATTCTGTAAGTTG  
1981 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
CCGGGTCCTTATACGTAAAAATTTTTCAGTAGACGGGAAGGGTCCACTAAGACATTCAAC 2040  
TCCCTCAACTGTACTTGGAGAAATCGTGTTTTTAAAGCAGTAGTCCACAAAGTATTCTGCT  
2041 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
AGGGAGTTGACATGAACCTCTTTAGCACAAAATTTTCGTCATCAGGTGTTTCATAAGACGA 2100  
CATGTGCCCCCAAAGTATTTTGAAAAATCATGTATACCCTCACCCATCTAAGTTGATAT  
2101 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
GTACACGGGGGTTTTTCATAAACTTTTTAGTACATATGGGAGTGGGTAGATTCAACTATA 2160  
CTAAAAATTTTATCTAAGTTGGTATCTAAAATTTTTTCATGGGAAGTTAAATAGTTGACAAA  
2161 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
GATTTTAAATAGATTCAACCATAGATTTTAAAAAGTACCCTTCAATTTATCAACTGTTT 2220  
GTATGTATTTGCTGGTGTCTGTAAATATTGGTATTTTAAAAATAAAACTGTTACATCAC  
2221 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
CATAcataAACGACCACAGCACATTTATAACCATAAAATTTTATTTTGGACAATGTAGTG 2280  
TAAWWWAAAAAAAAAAAAAAAAAAAAA  
2281 -----+-----+-----+-----+-----+-----+-----+-----+-----+-----+  
ATTWWTTTTTTTTTTTTTTTTTTTTT 2306



9/22

Figure 5. Coding region of human PSKB cDNA sequence.

```

38  ATGACGGTGTCTTTAAACGCTTCGAAATCACTGGAAGAAACTACAGCTGGGCTCTGC
    -+-----+-----+-----+-----+-----+-----+-----+-----+
97  TACTGCCACAAGAAATTTTGCGAAGCTTTAGTGACCTTCTTTTGATGTCGACCCGAGACG
    -+-----+-----+-----+-----+-----+-----+-----+-----+
158 CTGCTGACCTGGGGAGGCCATTGGCTCTATGGAACACTGTGATAACCTCCTAAGGAGA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
157 GACGACTGGACCCCTCCGGTAACCGAGATACCTTTTGTGACACTATTGGAGGATTCTCT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
158 GCAGCCTGTCAAGAAGCTCAGGTGTTTGGCAATCAACTCATTCTCCCAATGCACAAGTG
    -+-----+-----+-----+-----+-----+-----+-----+-----+
217 CGTCGGACAGTTCTTCGAGTCCACAAACCGTTAGTTGAGTAAGGAGGGTTACGTGTTTAC
    -+-----+-----+-----+-----+-----+-----+-----+-----+
218 AAGAAGGCCACTGTTTTCTCAATCCTGCAGCTTGCAAAGGAAAAGCCAGGACTCTATTT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
277 TTCTTCCGGTGACAAAAGAGTTAGGACGTCGAACGTTTCTTTTCGGTCTGAGATAAA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
278 GAAAAAATGCTGCCCCGATTTTACATTTATCTGGCATGGATGTGACTATTGTTAAGACA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
337 CTTTTTTTACGACGGGGCTAAAATGTAAATAGACCGTACCTACACTGATAACAATTCTGT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
338 GATTATGAGGGACAAGCCAAGAACTCCTGGAAGTATGGAACACGGATGTGATCATT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
397 CTAATACTCCCTGTTTCGGTTCCTTGAGGACCTTGACTACCTTTTGTGCCTACACTAGTAA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
398 GTTGCAGGAGGAGATGGGACACTGCAGGAGGTTGTTACTGGTGTCTTCGACGAACAGAT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
457 CAACGTCCTCCTCTACCCTGTGACGTCCTCCAACAATGACCACAAGAAGCTGCTTGTCTA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
458 GAGGCTACCTTCAGTAAGATTCCCATTTGATTATCCCACTGGGAGAGACCAGTAGTTTG
    -+-----+-----+-----+-----+-----+-----+-----+-----+
517 CTCCGATGGAAGTCATTCTAAGGGTAACCTAAATAGGGTGACCCCTCTCTGGTCATCAAAC
    -+-----+-----+-----+-----+-----+-----+-----+-----+
518 AGTCATACCCCTCTTTGCCGAAAGTGGAACAAAGTCCAACATATTACTGATGCCACACTT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
577 TCAGTATGGGAGAAACGGCTTTCACCTTTGTTTCAGGTTGTATAATGACTACGGTGTGAA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
578 GCCATTGTGAAAGGAGAGACAGTTCCACTTGATTCTTGCAGATCAAGGGTGAAAAGGAA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
637 CGGTAACACTTTCCTCTCTGTCAAGGTGAAGTAAAGAACGTCTAGTTCCCACTTTTCCTT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
638 CAGCCTGTATTTGCAATGACCGGCCTTCGATGGGGATCTTTCAGAGATGCTGGCGTCAAA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
697 GTCGGACATAAACGTTACTGGCCGGAAGCTACCCCTAGAAAGTCTCTACGACCGCAGTTT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
698 GTTAGCAAGTACTGGTATCTTGGGCCTCTAAAAATCAAAGCAGCCCACTTTTTCAGCACT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
757 CAATCGTTCATGACCATAGAACCCGGAGATTTTATGTTTCGTCGGGTGAAAAAGTCGTGA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
758 CTTAAGGAGTGGCCTCAGACTCATCAAGCCTCTATCTCATACACGGGACCTACAGAGAGA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
817 GAATTCCTCACCAGGAGTCTGAGTAGTTCGGAGATAGAGTATGTGCCCTGGATGTCTCTCT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
818 CCTCCCAATGAACCAGAGGAGACCCCTGTACAAAGGCCTTCTTTGTACAGGAGAATATTA
    -+-----+-----+-----+-----+-----+-----+-----+-----+
877 GGAGGGTTACTTGGTCTCCTCTGGGGACATGTTTCCGGAAGAAACATGTCTCTTATAAT
    -+-----+-----+-----+-----+-----+-----+-----+-----+
878 CGAAGGCTTGCGTCTACTGGGCACAACCACAGGATGCCCTTTCCCAAGAGGTGAGCCCG
    -+-----+-----+-----+-----+-----+-----+-----+-----+
937
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10/22

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11/22

Figure 6. Predicted polypeptide sequence of human PSKB protein.

1	MTVFFKTLRHNWKTTAGLCLLTWGGHWWLYGKHCDNLLRRAACQEAQVFGNQLIPPNAQV	60
61	KKATVFLNPAACKGKARTLFEKNAAPILHLSGMDVTIVKTDYEGQAKLLELMENTDVII	120
121	VAGGDGTLQEVVTGVLRRRTDEATFSKIPIGFIPLGETSSLSHTLFAESGNKVQHITDATL	180
181	AIVKGETVPLDFLQIKGEKEQPVFAMTGLRWGSFRDAGVKVSKYWYLGPLKIKAAHFFST	240
241	LKEWPQTHQASISYTGPTERPPNEPEETPVQRPSPYLRILRRLASYWAQPDALSQEVSP	300
301	EVWKDVQLSTIELSITTRNNQLDPTSKEDFLNICIEPDTISKGDFITIGSRKVRNPKLHV	360
361	EGTECLQASQCTLLIPEGAGGSFSIDSEYEAMPVEVKLLPRKLQFFCDPRKREQMLTSP	420
	TQ*	
421	---	423

Figure 7. Full-length human PSKC cDNA sequence.

**SUBSTITUTE SHEET (RULE 26)**

Figure 7 cont'd

13/22

781 AGGTTTAAAGACCTTCCTCTCCCACTGCTATGAAGGGACAGTGTCTTCTCCCTGC  
-----+-----+-----+-----+-----+-----+-----+-----+  
TCCAAATTTCTGGAAGGAGAGGGTGGTGACGATACTTCCCTGTACAGGAAGGAGGGACG 840

841 ACAACACACGGTGGGATCTCCAAGGGATAGGAAGCCCTGCCGGGCAGGATGCTTTGTTTG  
-----+-----+-----+-----+-----+-----+-----+-----+  
TGTTGTGTGCCACCCTAGAGGTTCCCTATCCTTCGGGACGGCCCGTCCTACGAAACAAAC 900

901 CAGGCAAAGCAAGCAGCAGCTGGAGGAGGAGCAGAAGAAAGCACTGTATGGTTTGGAAGC  
-----+-----+-----+-----+-----+-----+-----+-----+  
GTCCGTTTCGTTTCGTCTCGACCTCCTCCTCGTCTTCTTTCGTGACATACCAAACCTTCG 960

961 TGCGGAGGACGTGGAGGAGTGGCAAGTCTGTGTGGGAAGTTTCTGGCCATCAATGCCAC  
-----+-----+-----+-----+-----+-----+-----+-----+  
ACGCCTCCTGCACCTCCTCACCGTTTCAGCAGACACCCTTCAAAGACCGGTAGTTACGGTG 1020

1021 AAACATGTCCTGTGCTTGTGCGCGGAGCCCCAGGGGCCTCTCCCCGGCTGCCCACTTGGG  
-----+-----+-----+-----+-----+-----+-----+-----+  
TTTGTACAGGACACGAACAGCGGCCTCGGGGTCCCCGGAGAGGGGCCGACGGGTGAACCC 1080

1081 AGACGGGTCTTCTGACCTCATCCTCATCCGAAATGCTCCAGGTTCAATTTTCTGAGATT  
-----+-----+-----+-----+-----+-----+-----+-----+  
TCTGCCCAGAAGACTGGAGTAGGAGTAGGCCTTTACGAGGTCCAAGTTAAAGACTCTAA 1140

1141 TCTCATCAGGCACACCAACCAGCAGGACCAGTTTGACTTCACTTTTGTGGAAGTTTATCG  
-----+-----+-----+-----+-----+-----+-----+-----+  
AGAGTAGTCCGTGTGGTTGGTCTGCTCAAAGTGAAGTGAAAACAACCTTCAAATAGC 1200

1201 CGTCAAGAAATTCCAGTTTACGTGCAAGCACATGGAGGATGAGGACAGCGACCTCAAGGA  
-----+-----+-----+-----+-----+-----+-----+-----+  
GCAGTTCTTTAAGGTCAAATGCAGCTTCGTGTACCTCCTACTCCTGTGCTGGAGTTCTT 1260

1261 GGGGGGAAGAAGCGCTTTGGGCACATTTCAGCAGCCACCCCTCCTGCTGCTGCACCGT  
-----+-----+-----+-----+-----+-----+-----+-----+  
CCCCCCTTCTTCGCGAAACCCGTGTAAACGTCTGCGGTGGGGAGGACGACGACGTGGCA 1320

1321 CTCCAACAGCTCCTGGAAGTGGCAGCGGGAGGTCCTGCACAGCCCTGCCATCGAGGTCAG  
-----+-----+-----+-----+-----+-----+-----+-----+  
GAGGTTGTGAGGACCTTGACGCTGCCCCCTCAGGACGTGTGCGGACGGTAGCTCCAGTC 1380

1381 AGTCCACTGCCAGCTGGTTCGACTCTTTCGACGAGAATTGGAAGAGAATCCGAAGCCAGA  
-----+-----+-----+-----+-----+-----+-----+-----+  
TCAGGTGACGGTCGACCAAGCTGAGAAACGTGCTCTTAACCTTCTCTTAGGCTTCGGTCT 1440

1441 CTCACACAGCTGAGAAGCCGGCGTCTGCTCTCGAACTGGGAAAGTGTGAAAACCTATTTA  
-----+-----+-----+-----+-----+-----+-----+-----+  
GAGTGTGTGCGACTCTTCGGCCGAGGACGAGAGCTTGACCCTTTCACACTTTTGATAAAT 1500

1501 AGATAATTATTACAGACCAATTATGTTGATATATACATTTAAATGTAGAAATTTATTTTT  
-----+-----+-----+-----+-----+-----+-----+-----+  
TCTATTAATAATGTCTGGTTAATACTATATATGTAAATTTACATCTTTAAATAAAAA 1560

1561 GATAGTTAAATCTTGATTTTAGAAGAAAACCCCTTTGTCAACAATTTTGTGTACATATTT  
-----+-----+-----+-----+-----+-----+-----+-----+  
CTATCAATTTAGAACTAAAATCTTCTTTTGGGAAAACAGTTGTTAAAACACATGTATAAA 1620

Figure 7 cont'd

14/22

GGCATTTCAGTTCTGTACGCATCTGCGGGTTGCAGCCACGCGCTTACTCTCAGCGGA  
1621 -----+-----+-----+-----+-----+-----+-----+ 1680  
CCGTAAAAGTCAAGACATGCGTAGACGCCAACGTCGGGTGCGGCGAATGAGAGTCGCTT

TGCAGCTGCTCACTTGGGGGCACTGGCCTCTTAGGTTTTAACGATGTCAACAGTGTAGTT  
1681 -----+-----+-----+-----+-----+-----+-----+ 1740  
ACGTCGACGAGTGAACCCCCGTGACCGGAGAATCCAAAATTGCTACAGTTGTCACATCAA

TAGAAAATGGCCCGTTAGTGGCTCTATTGCAATAATGTTAGGGACATTATATGATTTCCA  
1741 -----+-----+-----+-----+-----+-----+-----+ 1800  
ATCTTTTACCGGGCAATCACCGAGATAACGTTATTACAATCCCTGTAATATACTAAAGGT

CGCAGGTCACACCATCTGGGCCTGAGGTAGCAGTGGGTCACTTTGATCCACTTTGCAGGA  
1801 -----+-----+-----+-----+-----+-----+-----+ 1860  
GCGTCCAGTGTGGTAGACCCGGACTCCATCGTCACCCAGTGAACTAGGTGAAACGTCCT

CTTATTCTGTAACGGTTTGTGGCCAAGTTTTGGGAAGTGGTTGATTCTCTTTGCCTTCAT  
1861 -----+-----+-----+-----+-----+-----+-----+ 1920  
GAATAAGACATTGCCAAACACCGGTTCAAAACCCCTTCACCACTAAGAGAAACGGAAGTA

TTCACCTTCCTCTTCGTTTACGGTTAGGACATCGCTGCTTGATCCTTACAATACTGTGCA  
1921 -----+-----+-----+-----+-----+-----+-----+ 1980  
AAGTGGAAAGGAGAAGCAAATGCCAATCCTGTAGCGACGAAGTAGGAATGTTATGACACGT

ACTGCAATGCAACGTGGCCCTGCTTCAGGTGATCCGCGGGAGGGGCTCCACGCCAGCAC  
1981 -----+-----+-----+-----+-----+-----+-----+ 2040  
TGACGTTACGTTGCACCGGGACGAAGTCCACTAGGCGCCCTCCCCGGAGGTGCGGTGCTG

C GGGAAAGGCTGCTGGGGCCTCCACACCTGCCTCATCACGGGGGGGAAGCTACGACAATC  
2041 -----+-----+-----+-----+-----+-----+-----+ 2100  
GCCCTTTCGACGACCCCGGAGGTGTGGACGGAGTAGTGCCCCCCTTCGATGCTGTTAG

CGGCTGGGAACATGACCTTGGCGTCTGTTCTGGGAACACAAATRATAARCTCTGGAARCT  
2101 -----+-----+-----+-----+-----+-----+-----+ 2160  
GCCGACCCTTGTACTGGAACCGCAGACAAGACCCTTGTGTTTAYTATTYAGACCTTYGA

GGCAGTGTGTAAAGCACTGGCAAGTTTGTACTGTAAAATGTCAAATACCAATGCTTTA  
2161 -----+-----+-----+-----+-----+-----+-----+ 2220  
CCGTCACACATTTCTGTACCGTTCAAACAATGACAATTTTACAGTTTATGGTTACGAAAT

TATCGACGCCGAAATGCTTAACACAKCCGGGCTTGGGGGAGTCAGGAAGAAAAGTGGCC  
2221 -----+-----+-----+-----+-----+-----+-----+ 2280  
ATAGCTGCGGCTTTACGAATTGTGTMGGCCCGAACCCCGTCAGTCCTTCTTTTGACCGG

ATCCGTGGAGGAGGGGCCGTCTGGACTCCCGCAGGAYTCCTCTGATGCAGGGCCTGAA  
2281 -----+-----+-----+-----+-----+-----+-----+ 2340  
TAGGCACCTCCTCCCCGGCCAGGACCTGAGGGCGTCTRAGGAGACTACGTCCCGGACTT

GTCTGTACACGTGGTCCAAATTTGTCCTTGTCTTTCTTCACACTGAGTTCTCTATATTT  
2341 -----+-----+-----+-----+-----+-----+-----+ 2400  
CAGACATGTGCACCAGGTTTAAACAGGAACAGAAAAGAAGTGTGACTCAAGAGATATAAA

ATTGAACATCTTGTCTTTTAAARCCAMGAAGTARTGTAACTGCGTCTCGGATGTCTGT  
2401 -----+-----+-----+-----+-----+-----+-----+ 2460  
TAACCTGTAGAACAGGAAAAATTYGGTKCTTCATYACAATTGACGCAGAGCCTACAGACA

Figure 7 cont'd

15/22

2461 CTTTTGTCTCSGAARCCACRAWGGATCKCTGGTTTCTCTCTGTCAGCGCGAGGGCTCCGG  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2520  
GAAACAGAGSCTTYGGTGYTWCCTAGMGACCAAAGGAGAGACGTGCGCTCCCCGAGGCC

2521 CGACCAGAGGATYCTYCCCGRAAGGSATTCTGCGCGCTCCCCCGGGCACCCCTCAATT  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2580  
GCTGGTCTCCTARGARGGGCYTTCSTAAAGACGGCGCGAGGGGGCCCGTGGGGAGTTAA

2581 GTGTACTACCGTCCCTTGTTTAAKGGTTTGTATCCCTGCCCACSTAAGATAAATGTCTGT  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2640  
CACATGATGGCAGGGAACAAATTMCCAAACATAGGGACGGGTGSATTCTATTACAGACA

2641 AACGGTAGTTTTGTTTAAAAATATGAGAATATGCGGCTTAACTTTGATCTGTAAGGAGC  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2700  
TTGCCATCAAAACAACTTTTATACTCTTATACGCCGAATTTGAACTAGACATTCTCG

2701 GGGGCCCCGTGCCCCGTTTGGAGCACGCTGTAGACMCCGTTCTCATGCTGCCGGGTGGGTT  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2760  
CCCCGGGCACGGGCAAACCTCGTGCGACATCTGKGGCAAGGAGTACGACGCCCCACCCAA

2761 TTGCAGAAGCTCCCTTAGTGATTTTCATGTTTAAACAGGCAGCATCCCATTTTCAGAATTC  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2820  
AACGTCTTCGAGGAATCACTAAAGTACAAATTGTCGTCGTAGGGTAAAGTCTTAAAG

2821 CTGGCATTGATTTTATATTTTGAAGCATACAGGAACTTCTCGTTTCCCTCGTTTAGCCC  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2880  
GACCGTAACATAAAATATAAACTTCGTATGTCCTTTGAAGAGCAAAGGGAGCAAATCGGG

2881 CCACCCAGATCCAGGTGAAAGGGCAGCTTTAATGGTGGTTTTTATGGACCACCATTATCA  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2940  
GGTGGGTCTAGGTCCACTTTCCCGTCGAAATTACCACCAAAAATACCTGGTGGTAATAGT

2941 GAGAGCACTGTGCAAGCCAAATGGTTCCAATAATGAATGAAAATTTCTGGGTGTAAAGAG  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 3000  
CTCTCGTGACACGTTTCGGTTTACCAAGGTTATTACTTACTTTTAAAGACCCACATTCTC

3001 TAAATATGCCCTGGCTCTTTTCTACCAATGTTTGCTTCTCGGTTGGAAAGAAACCAAAG  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 3060  
ATTTATACGGGGACCGAGAAAAGATGGTTACAAACGAAGGACCAACCTTTCTTTGGTTTC

3061 ATTTAAGACGGGCTGCTTCTTCCAGACTGGCTGTGCCCTGCCTGTGGCCCAGCAACCTG  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 3120  
TAAATTCGCCCCGACGAAGAAGGGTCTGACCGACACGGGACGGACACCGGTCGTTGGAC

3121 TGCAGCCGGCAGTGTGCCTGGTGTACGCCAGGAGGCTGTGGCTGCTGTGGGCCCTCTGG  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 3180  
ACGTCGGCCGTCACACGGACCACAGTGCGGTCTCCGACACCGACGACACCCGGGAGACC

3181 AATTGTGCTCCCTCCACAAAGTTTACCCCAAAGGTTCTTCTAAGCCTTTATTGTCCCCT  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 3240  
TTAACACGAGGGAGGTGTTTCAAATGGGGTTTTCCAAGAAGATTGCGAAATAACAGGGGA

3241 GGTAAATGTTTCCCTGGCTGGGCGCGGTGGCTCCACGCTGTAATCCAGCACTTTGGGA  
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 3300  
CCATTTACAAAGGGACCGACCCGCGCCACCGAGGTGCGGACATTAGGGTCGTGAAACCT

16/22

3301 GGCCGAGGCGGGTGGATCCACCTAAGGTGAGGAGTTTGAGATCCAGCCTGCCCAACATGG  
-----+-----+-----+-----+-----+-----+  
CCGGCTCCGCCCACCTAGGTGGATTCCAGTCCTCAAACCTCTAGGTCGGACGGGTTGTACC 3360

3361 TGAAACCTYGTCTTCTACTAAAAATACACAACCTTAGCCAGTCTTGTTGGCGMACGCCTGTA  
-----+-----+-----+-----+-----+-----+  
ACTTTGGARCAAAGATGATTTTATGTGTTGAATCGGTCAGAACAAACCGCKTGCGGACAT 3420

3421 ATSTTCAGYTACTAGGGACGCTGAGGCAGGAGAATCGTTTGAACCCAAGAAAGAGGTGGA  
-----+-----+-----+-----+-----+-----+  
TASAAGTCRATGATCCCTGCGACTCCGTCCTCTTAGCAAACCTTGGGTTCTTTCTCCACCT 3480

3481 GGTGTVGGTGAGCCAAGATTGCGCCAHTGCACTCCAGCCTGGGCAACAGAGGGAGAYTCC  
-----+-----+-----+-----+-----+-----+  
CCAACBCCACTCGGTTCTAACGCGGTDACGTGAGGTCGGACCCGTTGTCTCCCTCTRAGG 3540

3541 ATCGCCCCCCCCCAACAAAAAAGTTTCCCATACAYTGGCSTGCCCCAAAACCCACT  
-----+-----+-----+-----+-----+-----+  
TAGCGGGGGGGGTTGTTTTTTTTTCAAAGGATGTRACCGSACGGGGTTTTGGGTGA 3600

3601 AACAAATTTTAGCAAAACAGTCCAGGCCAAAGAGGAAGCATTTYATGTTCAATAAGAAACC  
-----+-----+-----+-----+-----+-----+  
TTGTTAAATCGTTTTGTGTCAGGTCCGGTTTCTCCTTCGTAAARTACAAGTTATTCTTTGG 3660

3661 CAGCCATTCCGCATGGCTGGTTCCTGAGTGGCTYTGGTGATACTCTCCAGCCACCTGCTG  
-----+-----+-----+-----+-----+-----+  
GTCGGTAAGGCGTACCGACCAAGGACTCACCGARACCACTATGAGAGGTCGGTGGACGAC 3720

3721 ACATTGAGAATTTTACAGACYTCGGGACTGCTGTTGCGGTACCGTGTGTGTGACACCTGCCA  
-----+-----+-----+-----+-----+-----+  
TGTAATCTTTAAAGTCTGRAGCCCTGACGACAACGCCATGGCACACARACTGTGGACGGT 3780

3781 GCAGCCCTTTTGCTATTTGCGCGCAGGATGGGGGTGACTGCCCAGACATTCCCGCTAGATA  
-----+-----+-----+-----+-----+-----+  
CGTCGGGAAACGATAAACGCGCGTCTACCCCCACTGACGGGTCTGTAAGGGCGATCTAT 3840

3841 GGTTTTGATTTCCGGGGCAGCCTTTTTCAGATGCGGCAGACATACAACACCTGTACTTTAGA  
-----+-----+-----+-----+-----+-----+  
CCAAAATAAAGGCCCGTCGGAAAGTCTACGCCGTCTGTATGTTGTGGACATGAAATCT 3900

3901 GTTTTAAGGGAAAAAATCAGAAGTGCTGGTTAGATAGTAAAACTTAGGATAACTTA  
-----+-----+-----+-----+-----+-----+  
CAAAATTCCTTTTTTTTTTAGTCTTCACGACCAATCTATCATTTTTGAATCCTATTGAAT 3960

3961 GAAAGGCTAGTTTTAGCTTCCTTTGTGGCTCCCCTGGTGCAAAACAATTAGCAGTTATGC  
-----+-----+-----+-----+-----+-----+  
CTTTCGGATCAAAATCGAAGGAAACACCGAGGGGACCACGTTTTGTTAATCGTCAATACG 4020

4021 AATGGACCTGATTCTAGTTTATTCTAATTAAGAAGTGAGGCCGGGTTTGRACTTCGTTCC  
-----+-----+-----+-----+-----+-----+  
TTACCTGGACTAAGATCAAATAAGATTAATTCTTCACCTCCGGCCCCAAACYTGAAGCAAGG 4080

4081 TGAATACAATCTTGAGTAACTGGGAAAGTCTGAGTGAAAGGATGGCCTCATTCTCTTTCT  
-----+-----+-----+-----+-----+-----+  
ACTTATGTTAGAATCATTGACCCCTTCAGACTCACTTTCCTACCGGAGTAAGAGAAAGA 4140



Figure 7 cont'd

17/22

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4141 AATCTTGCTGGTTTCAAGATTAGAAAATGGCATTATTTGATCTGAAATGTTTGAGAARAC
-----+-----+-----+-----+-----+-----+-----+
TTAGAACGACCAAAGTTCTAATCTTTTACCGTAATAAACTAGACTTTACAAACTCTTYTG 4200

ACGAATAAAGTTACTTGGGCAGAAAAAAAAA
4201 -----+-----+-----+-----+-----+-----+-----+
TGCTTATTTCAATGAACCCGTCTTTTTTTTT 4231
```

18/22

Figure 8. Coding region of human PSKC cDNA.

	ATGGAAAAGCCTTACGCTTTTACAGTTCACTGTGTAAAGAGAGCACGACGGCACCGCTGG	
71	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	130
	TACCTTTTCGGAATGCGAAAATGTCAAGTGACACATTCTCTCGTGCTGCCGTGGCGACC	
	AAGTGGGCGCAGGTGACTTTCTGGTGTCCAGAGGAGCAGCTGTGTCACTTGTGGCTGCAG	
131	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	190
	TTCACCCGCGTCCACTGAAAGACCACAGGTCTCCTCGTCGACACAGTGAACACCGACGTC	
	ACCCTGCGGGAGATGCTGGAGAAGCTGACGTCCAGACCAAAGCATTTACTGGTATTTATC	
191	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	250
	TGGGACGCCCTCTACGACCTCTTCGACTGCAGGTCTGGTTTTCGTAAATGACCATAAATAG	
	AACCCGTTTGGAGGAAAAGGACAAGGCAAGCGGATATATGAAAGAAAAGTGGCACCCTG	
251	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	310
	TTGGGCAAACCTCCTTTTCTGTTCGGTTCGCCTATATACTTTCTTTTCACCGTGGTGAC	
	TTCACCTTAGCCTCCATCACCCTGACATCATCGTTACTGAACATGCTAATCAGGCCAAG	
311	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	370
	AAGTGGAAATCGGAGGTAGTGGTGACTGTAGTAGCAATGACTTGTACGATTAGTCCGGTTC	
	GAGACTCTGTATGAGATTAACATAGACAAATACGACGGCATCGTCTGTGTGCGCGGAGAT	
371	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	430
	CTCTGAGACATACTCTAATTGTATCTGTTTATGCTGCCGTAGCAGACACAGCCGCTCTA	
	GGTATGTTTACGCGAGGTGCTGCACGGTCTGATTGGGAGGACGCAGAGGAGCGCCGGGGTC	
431	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	490
	CCATACAAGTCGCTCCACGACGTGCCAGACTAACCCCTCCTGCGTCTCCTCGCGCCCCAG	
	GACCAGAACCACCCCCGGGCTGTGCTGGTCCCCAGTAGCCTCCGGATTGGAATCATTCCC	
491	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	550
	CTGGTCTTGGTGGGGGCCCCGACACGACCAGGGGTCATCGGAGGCCTAACCTTAGTAAGGG	
	GCAGGGTCAACGGACTGCGTGTGTTACTCCACCGTGGGCACCAGCGACGCAGAAACCTCG	
551	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	610
	CGTCCCAGTTGCTGACGCACACAATGAGGTGGCACCCGTTGGTTCGCTGCGTCTTTGGAGC	
	GCGCTGCATATCGTTGTTGGGGACTCGCTGGCCATGGATGTGTCCTCAGTCCACCACAAC	
611	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	670
	CGCGACGTATAGCAACAACCCCTGAGCGACCGGTACCTACACAGGAGTCAGGTGGTGTG	
	AGCACACTCCTTCGCTACTCCGTGTCCCTGCTGGGCTACGGCTTCTACGGGGACATCATC	
671	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	730
	TCGTGTGAGGAAGCGATGAGGCACAGGGACGACCCGATGCCGAAGATGCCCTGTAGTAG	
	AAGGACAGTGAGAAGAAACGGTGGTTGGGTCTTGCCAGATACGACTTTTCAGGTTTAAAG	
731	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	790
	TTCTGTCACTCTTCTTTGCCACCAACCCAGAACGGTCTATGCTGAAAAGTCCAAATTTT	
	ACCTTCCTCTCCCACTGCTATGAAGGGACAGTGTCTTCTCCTCCCTGCACAACACACG	
791	- - - - - + - - - - - + - - - - - + - - - - - + - - - - - +	850
	TGGAAGGAGAGGGTGGTGACGATACTTCCCTGTACAGGAAGGAGGGACGTGTTGTGTGC	

Figure 8 cont'd

19/22

851 GTGGGATCTCCAAGGGATAGGAAGCCCTGCCGGGCAGGATGCTTTGTTTGCAGGCAAAGC  
-----+-----+-----+-----+-----+-----+-----+-----+-----+ 910  
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911 AAGCAGCAGCTGGAGGAGGAGCAGAAGAAAGCACTGTATGGTTTGGAAAGCTGCGGAGGAC  
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TTCGTCGTCGACCTCCTCCTCGTCTTCTTCGTGACATACCAAACCTTCGACGCCTCCTG

971 GTGGAGGAGTGGCAAGTCGTCTGTGGGAAGTTTCTGGCCATCAATGCCACAAACATGTCC  
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CACCTCCTCACCGTTCAGCAGACACCCCTCAAAGACCGGTAGTTACGGTGTGTTGTACAGG

1031 TGTGCTTGTGCGCCGAGCCCCAGGGGCTCTCCCCGGCTGCCCACTTGGGAGACGGGTCT  
-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1090  
ACACGAACAGCGGCCTCGGGGTCCCCGGAGAGGGGCCGACGGGTGAACCCTCTGCCCAGA

1091 TCTGACCTCATCCTCATCCGAAATGCTCCAGGTTCAATTTTCTGAGATTTCTCATCAGG  
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AGACTGGAGTAGGAGTAGGCCTTTACGAGGTCCAAGTTAAAGACTCTAAAGAGTAGTCC

1151 CACACCAACCAGCAGGACCAGTTTGACTTCACTTTGTGTAAGTTTATCGCGTCAAGAAA  
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GTGTGGTTGGTCGTCCTGGTCAAACCTGAAGTGAACAACCTTCAAATAGCGCAGTTCTTT

1211 TTCCAGTTTACGTCGAAGCACATGGAGGATGAGGACAGCGACCTCAAGGAGGGGGGAAG  
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1271 AAGCGCTTTGGGCACATTTGCAGCAGCCACCCCTCCTGCTGCTGCACCGTCTCCAACAGC  
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1331 TCCTGGAAGTGGACGGGGAGGTCCTGCACAGCCCTGCCATCGAGGTCAGAGTCCACTGC  
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AGGACCTTGACGCTGCCCCCTCAGGACGTGTGCGGACGGTAGCTCCAGTCTCAGGTGACC

1391 CAGCTGGTTGACTCTTTGCACGAGAATTGGAAGAGAATCCGAAGCCAGACTCACACAGC  
-----+-----+-----+-----+-----+-----+-----+-----+-----+ 1450  
GTCGACCAAGCTGAGAAACGTGCTCTTAACCTTCTCTTAGGCTTCGGTCTGAGTGTGTCG

TGA  
1451 --- 1453  
ACT

20/22

Figure 9. Predicted amino acid sequence of human PSKC protein.

```
1  MEKPYAFTVHCVKRARRHRWKWAQVTFWCPEEQQLCHLWLQTLREMLEKLTSRPKHLLVFI  60
   -----+-----+-----+-----+-----+-----+-----+
61  NPFGGKGQGKRIYERKVAPLFTLASITTDIIVTEHANQAKETLYEINIDKYDGIVCVGGD  120
   -----+-----+-----+-----+-----+-----+-----+
121 GMFSEVLHGLIGRTQRSAGVDQNHPRAVLVPSSLRIGIIPAGSTDCVCYSTVGTSDAETS  180
   -----+-----+-----+-----+-----+-----+-----+
181 ALHIVVGDSLAMDVSSVHHNSTLLRYSVSLGFGFYGDIKDSEKKRWLGLARYDFSGLK  240
   -----+-----+-----+-----+-----+-----+-----+
241 TFLSHHCYEGTVSFLPAQHTVGSPRDRKPCRAGCFVCRQSKQOLEEEQKKALYGLEAAED  300
   -----+-----+-----+-----+-----+-----+-----+
301 VEEWQVVCCKFLAINATNMSCACRRSPRGLSPAHLGDGSSDLILIRKCSRFNFLRFLIR  360
   -----+-----+-----+-----+-----+-----+-----+
361 HTNQDQDFDTFVEVYRVKKFQFTSKHMEDESDLKEGGKKRFGHICSSHPSCCCTVSNS  420
   -----+-----+-----+-----+-----+-----+-----+
421 SWNCDGEVLHSPAIEVRVHCQLVRLFARELEENPKPDHS*  461
   -----+-----+-----+-----+-----+-----+-----+
```

Figure 10

21/22

**Multiple alignment of novel human SK-like amino acid sequences.**

```

PSKA_Human  -----MDPAGGPRGVLP---RP
PSKC_Human  MEKPYAFTVHCVKRARR---HRWKWAQVTFWCPEEQCHLWLQTLREMLEKLT---RP
PSKB_Human  MTVFFKTLRNHWKKT TAGLCLLTWGGHWLYGKHCDNLLRRAACQEAQVFGNQLIPPNAQV

PSKA_Human  CRVLVLLNPRGGKGKALQLF RSHVQPLLA EAEISFTLMLTERRNHARELVRSEELGRWDA
PSKC_Human  KHLVFINPFGGKGQGKRIYERKVAPLFTLASITTDIIIVTEHANQAKETLYEINIDKYDG
PSKB_Human  KKATVFLNPAACKGKARTLFEKNAAPILHLSGMDVTIVKTDYEQAKKLL--ELMENTDV
      . * . **   ** . . . . * . . . . * . . . . *

PSKA_Human  LVVMSGDGLMHEVVNGLMERPDWETAIQK--PLCILPAGSG--NALAASLNHYAGYEQVT
PSKC_Human  IVCVGGDGMFSEVLHGLIGRTQRSAGVDQNHPRAVLVPSSLRIGIIPAGSTDCVCYSTVG
PSKB_Human  IIVAGGDGTLQEVVTGVLRTDEATFSKI--PIGFIPGET-----SSLSHTLFAESGN
      .. ***   ** . * . . . . * . . . .

PSKA_Human  NEDLLTNCTLLLCRRLSPMNL LSLHTASGLRLFSVLSLAWGFIADV DLESEKYRRLGEM
PSKC_Human  TSDAETSALHIVVGDSL A-MDVSSVHHNSTLLRYSVSL LGYGFYGDIIKDSEKKRWLGLA
PSKB_Human  KVQHITDATLAIVKGETVPLD FLQIKGEKEQPVFAMTGLRWGSFRDAGVKVSKYWLGPL
      * . . . . . . . . . * * * * *

PSKA_Human  RFTLTGTFRLAALR--TYRGRLAYLPVGR-VGSKTPASPVVVQ---QGPVDAHLVPLEEP
PSKC_Human  RYDFSGLKTFLSHH--CYEGTVSFLPAQHTVGSPDRKPCRAG---CFVCRQSKQOLEEE
PSKB_Human  KIKAAHFFSTLKEWPQTHQASISYTGPTERPPNEPEETPVQRPSLYRRILRRLASYWAQP
      . . . . . *

PSKA_Human  VP-----SHWTVVPDEDFVLVLALLHSHLG--SEMFAAPMGRCAAGVMHLFYV
PSKC_Human  QKKALYGLEAAEDVEEWQVVCG-KFLAINATNMSCACRRSPRGLSPAHLGDGSSDLILI
PSKB_Human  QD-----ALSQEVSPVEWKDVQLSTIELSITR--NNQLDPTSKED--FLNICIE
      . . . . * . . . .

PSKA_Human  RAGVSRAMLLRLFLAMEKGRHMEYECPYLVYVPVVAFRLEPKDGKG-VFAVDGELMVRRA
PSKC_Human  RK-CSRFNFLRFLIRHTN-QQDQDFTFVEVYRVKKFQFTSKHMEDESDLKEGGKKRFG
PSKB_Human  PDTISKGDFITIGSRKVR--NPKLHVEGTECLQASQCTLLIPEGAGGSF SIDSEYEAMP
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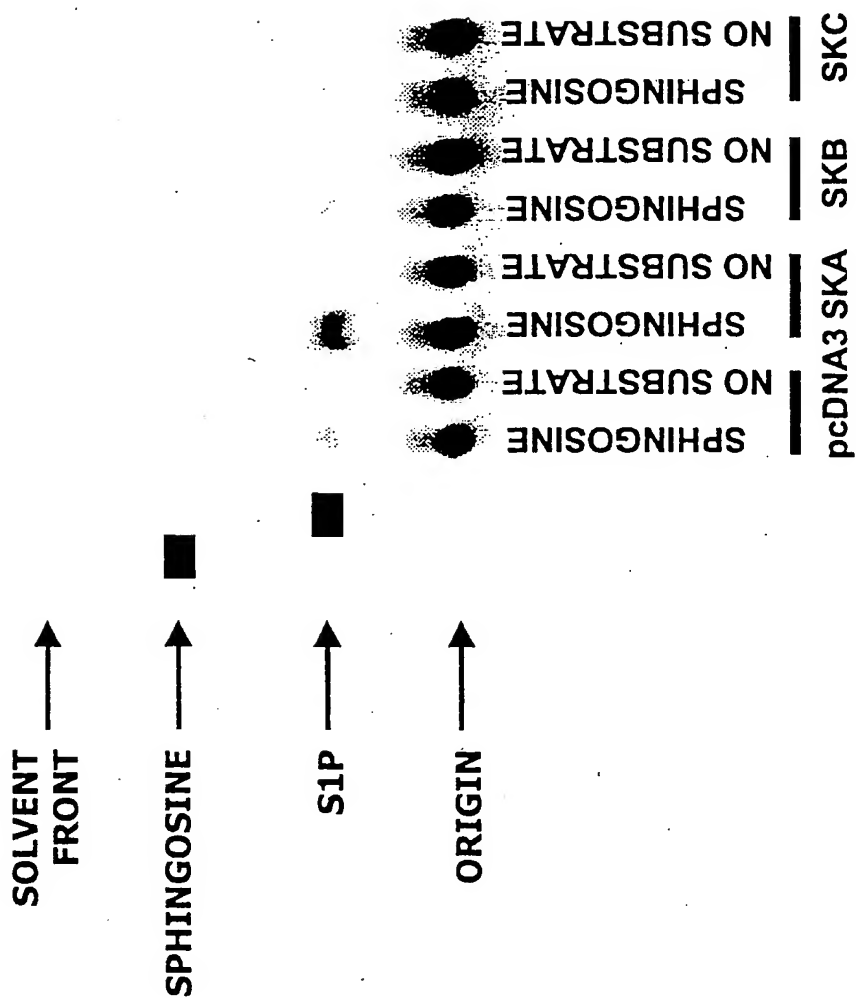
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PSKC_Human  HICSSHPSCCCTVSNSSWNCDGEVLHSPAIEVRVHCQLVRLFARELEENPKPDSHS
PSKB_Human  VEVKLLPR-----KLQFFCD-----PRKRE-----QMLTSPTQ--
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```

Figure 11

22/22

# Phosphorylation of Sphingosine by Cloned Human SK<sub>A</sub>



## SEQUENCE LISTING

<110> NPS Allelix Corp.

<120> HUMAN SPHINOSINE KINASE HOMOLOGUES

<130> p128pct3

<140> not yet issued

<141> 2000-03-02

<150> U.S. 60/122,516

<151> 1999-03-02

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<213> Homo sapiens

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cttttggtcg aggtgaaat ctcttcacg ctgatgctca ctgagcggcg gaaccacgcg 180
cgggagctgg tcgggtcgga ggagctgggc cgctgggacg ctctggtggt catgtctgga 240
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&lt;211&gt; 384

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 3

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```

```

Val Leu Val Leu Leu Asn Pro Arg Gly Gly Lys Gly Lys Ala Leu Gln
          20             25             30

```

```

Leu Phe Arg Ser His Val Gln Pro Leu Leu Ala Glu Ala Glu Ile Ser
      35             40             45

```

```

Phe Thr Leu Met Leu Thr Glu Arg Arg Asn His Ala Arg Glu Leu Val
      50             55             60

```

```

Arg Ser Glu Glu Leu Gly Arg Trp Asp Ala Leu Val Val Met Ser Gly
      65             70             75             80

```

```

Asp Gly Leu Met His Glu Val Val Asn Gly Leu Met Glu Arg Pro Asp
          85             90             95

```

```

Trp Glu Thr Ala Ile Gln Lys Pro Leu Cys Ile Leu Pro Ala Gly Ser
      100            105            110

```

```

Gly Asn Ala Leu Ala Ala Ser Leu Asn His Tyr Ala Gly Tyr Glu Gln
      115            120            125

```

```

Val Thr Asn Glu Asp Leu Leu Thr Asn Cys Thr Leu Leu Leu Cys Arg
      130            135            140

```

```

Arg Leu Leu Ser Pro Met Asn Leu Leu Ser Leu His Thr Ala Ser Gly
      145            150            155            160

```

```

Leu Arg Leu Phe Ser Val Leu Ser Leu Ala Trp Gly Phe Ile Ala Asp
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```

```

Val Asp Leu Glu Ser Glu Lys Tyr Arg Arg Leu Gly Glu Met Arg Phe
      180            185            190

```

```

Thr Leu Gly Thr Phe Leu Arg Leu Ala Ala Leu Arg Thr Tyr Arg Gly
      195            200            205

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 Leu Glu Glu Pro Val Pro Ser His Trp Thr Val Val Pro Asp Glu Asp  
 245 250 255  
 Phe Val Leu Val Leu Ala Leu Leu His Ser His Leu Gly Ser Glu Met  
 260 265 270  
 Phe Ala Ala Pro Met Gly Arg Cys Ala Ala Gly Val Met His Leu Phe  
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 Tyr Val Arg Ala Gly Val Ser Arg Ala Met Leu Leu Arg Leu Phe Leu  
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 305 310 315 320  
 Tyr Val Pro Val Val Ala Phe Arg Leu Glu Pro Lys Asp Gly Lys Gly  
 325 330 335  
 Val Phe Ala Val Asp Gly Glu Leu Met Val Arg Arg Ala Val Gln Gly  
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&lt;210&gt; 5

&lt;211&gt; 1269

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 5

```

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1269

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&lt;210&gt; 6

&lt;211&gt; 422

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 6

Met Thr Val Phe Phe Lys Thr Leu Arg Asn His Trp Lys Lys Thr Thr

1	5	10	15
Ala Gly Leu Cys Leu Leu Thr Trp Gly Gly His Trp Leu Tyr Gly Lys	20	25	30
His Cys Asp Asn Leu Leu Arg Arg Ala Ala Cys Gln Glu Ala Gln Val	35	40	45
Phe Gly Asn Gln Leu Ile Pro Pro Asn Ala Gln Val Lys Lys Ala Thr	50	55	60
Val Phe Leu Asn Pro Ala Ala Cys Lys Gly Lys Ala Arg Thr Leu Phe	65	70	75
Glu Lys Asn Ala Ala Pro Ile Leu His Leu Ser Gly Met Asp Val Thr	85	90	95
Ile Val Lys Thr Asp Tyr Glu Gly Gln Ala Lys Lys Leu Leu Glu Leu	100	105	110
Met Glu Asn Thr Asp Val Ile Ile Val Ala Gly Gly Asp Gly Thr Leu	115	120	125
Gln Glu Val Val Thr Gly Val Leu Arg Arg Thr Asp Glu Ala Thr Phe	130	135	140
Ser Lys Ile Pro Ile Gly Phe Ile Pro Leu Gly Glu Thr Ser Ser Leu	145	150	155
Ser His Thr Leu Phe Ala Glu Ser Gly Asn Lys Val Gln His Ile Thr	165	170	175
Asp Ala Thr Leu Ala Ile Val Lys Gly Glu Thr Val Pro Leu Asp Phe	180	185	190
Leu Gln Ile Lys Gly Glu Lys Glu Gln Pro Val Phe Ala Met Thr Gly	195	200	205
Leu Arg Trp Gly Ser Phe Arg Asp Ala Gly Val Lys Val Ser Lys Tyr	210	215	220
Trp Tyr Leu Gly Pro Leu Lys Ile Lys Ala Ala His Phe Phe Ser Thr	225	230	235
Leu Lys Glu Trp Pro Gln Thr His Gln Ala Ser Ile Ser Tyr Thr Gly	245	250	255
Pro Thr Glu Arg Pro Pro Asn Glu Pro Glu Glu Thr Pro Val Gln Arg	260	265	270
Pro Ser Leu Tyr Arg Arg Ile Leu Arg Arg Leu Ala Ser Tyr Trp Ala	275	280	285
Gln Pro Gln Asp Ala Leu Ser Gln Glu Val Ser Pro Glu Val Trp Lys	290	295	300
Asp Val Gln Leu Ser Thr Ile Glu Leu Ser Ile Thr Thr Arg Asn Asn	305	310	315
Gln Leu Asp Pro Thr Ser Lys Glu Asp Phe Leu Asn Ile Cys Ile Glu			

325                      330                      335  
 Pro Asp Thr Ile Ser Lys Gly Asp Phe Ile Thr Ile Gly Ser Arg Lys  
                          340                      345                      350  
 Val Arg Asn Pro Lys Leu His Val Glu Gly Thr Glu Cys Leu Gln Ala  
                          355                      360                      365  
 Ser Gln Cys Thr Leu Leu Ile Pro Glu Gly Ala Gly Gly Ser Phe Ser  
                          370                      375                      380  
 Ile Asp Ser Glu Glu Tyr Glu Ala Met Pro Val Glu Val Lys Leu Leu  
                          385                      390                      395                      400  
 Pro Arg Lys Leu Gln Phe Phe Cys Asp Pro Arg Lys Arg Glu Gln Met  
                          405                      410                      415  
 Leu Thr Ser Pro Thr Gln  
                          420

&lt;210&gt; 7

&lt;211&gt; 4231

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 7

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&lt;210&gt; 8

&lt;211&gt; 1383

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;400&gt; 8

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&lt;210&gt; 9

&lt;211&gt; 460

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 9

```

Met Glu Lys Pro Tyr Ala Phe Thr Val His Cys Val Lys Arg Ala Arg
  1             5             10             15

```

```

Arg His Arg Trp Lys Trp Ala Gln Val Thr Phe Trp Cys Pro Glu Glu
      20             25             30

```

```

Gln Leu Cys His Leu Trp Leu Gln Thr Leu Arg Glu Met Leu Glu Lys
      35             40             45

```

```

Leu Thr Ser Arg Pro Lys His Leu Leu Val Phe Ile Asn Pro Phe Gly
      50             55             60

```

```

Gly Lys Gly Gln Gly Lys Arg Ile Tyr Glu Arg Lys Val Ala Pro Leu
      65             70             75             80

```

```

Phe Thr Leu Ala Ser Ile Thr Thr Asp Ile Ile Val Thr Glu His Ala
      85             90             95

```

```

Asn Gln Ala Lys Glu Thr Leu Tyr Glu Ile Asn Ile Asp Lys Tyr Asp
      100            105            110

```

```

Gly Ile Val Cys Val Gly Gly Asp Gly Met Phe Ser Glu Val Leu His
      115            120            125

```

```

Gly Leu Ile Gly Arg Thr Gln Arg Ser Ala Gly Val Asp Gln Asn His
      130            135            140

```

```

Pro Arg Ala Val Leu Val Pro Ser Ser Leu Arg Ile Gly Ile Ile Pro
      145            150            155            160

```

```

Ala Gly Ser Thr Asp Cys Val Cys Tyr Ser Thr Val Gly Thr Ser Asp
      165            170            175

```

```

Ala Glu Thr Ser Ala Leu His Ile Val Val Gly Asp Ser Leu Ala Met
      180            185            190

```

```

Asp Val Ser Ser Val His His Asn Ser Thr Leu Leu Arg Tyr Ser Val
      195            200            205

```

```

Ser Leu Leu Gly Tyr Gly Phe Tyr Gly Asp Ile Ile Lys Asp Ser Glu
      210            215            220

```

```

Lys Lys Arg Trp Leu Gly Leu Ala Arg Tyr Asp Phe Ser Gly Leu Lys
      225            230            235            240

```

Thr	Phe	Leu	Ser	His	His	Cys	Tyr	Glu	Gly	Thr	Val	Ser	Phe	Leu	Pro
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			260					265					270		
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		275					280					285			
Lys	Lys	Ala	Leu	Tyr	Gly	Leu	Glu	Ala	Ala	Glu	Asp	Val	Glu	Glu	Trp
	290					295					300				
Gln	Val	Val	Cys	Gly	Lys	Phe	Leu	Ala	Ile	Asn	Ala	Thr	Asn	Met	Ser
305					310					315					320
Cys	Ala	Cys	Arg	Arg	Ser	Pro	Arg	Gly	Leu	Ser	Pro	Ala	Ala	His	Leu
				325					330					335	
Gly	Asp	Gly	Ser	Ser	Asp	Leu	Ile	Leu	Ile	Arg	Lys	Cys	Ser	Arg	Phe
			340					345					350		
Asn	Phe	Leu	Arg	Phe	Leu	Ile	Arg	His	Thr	Asn	Gln	Gln	Asp	Gln	Phe
		355					360					365			
Asp	Phe	Thr	Phe	Val	Glu	Val	Tyr	Arg	Val	Lys	Lys	Phe	Gln	Phe	Thr
	370					375					380				
Ser	Lys	His	Met	Glu	Asp	Glu	Asp	Ser	Asp	Leu	Lys	Glu	Gly	Gly	Lys
385					390					395					400
Lys	Arg	Phe	Gly	His	Ile	Cys	Ser	Ser	His	Pro	Ser	Cys	Cys	Cys	Thr
				405					410					415	
Val	Ser	Asn	Ser	Ser	Trp	Asn	Cys	Asp	Gly	Glu	Val	Leu	His	Ser	Pro
			420					425					430		
Ala	Ile	Glu	Val	Arg	Val	His	Cys	Gln	Leu	Val	Arg	Leu	Phe	Ala	Arg
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Glu	Leu	Glu	Glu	Asn	Pro	Lys	Pro	Asp	Ser	His	Ser				
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<211> 25

<212> DNA

<213> Artificial Sequence

**<220>**

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25

<210> 11

<211> 25

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:primer

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25

<210> 12  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:primer

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25

<210> 13  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:primer

<400> 13  
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24

<210> 14  
<211> 25  
<212> DNA  
<213> Artificial Sequence

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27

<210> 16  
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<223> Description of Artificial Sequence:primer

<400> 16



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27

<210> 17

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:primer

<400> 17

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23

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